

PATENT APPLICATION

QUINOLONES WITH ANTI-HIV ACTIVITY

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CROSS-REFERENCE TO RELATED APPLICATIONS

This is a non-provisional filing of U.S. Provisional Patent Application No. 60/420,163 filed on October 21, 2002, the disclosure of which is incorporated herein by
5 reference in its entirety for all purposes.

FIELD OF THE INVENTION

The present invention relates to inhibition of a virus, *e.g.*, HIV, using quinolone-based compounds. The compounds of the invention are of use to combat infection by mutant, drug-sensitive, drug-resistant, and multi-drug resistant strains of HIV. The
10 invention further relates to methods for identifying and using to inhibit HIV and to treat HIV-related diseases.

BACKGROUND OF THE INVENTION

The Human Immunodeficiency Virus (HIV) infects millions of people globally. Cases are reported from nearly every country amounting to 40 million adults and
15 children living with HIV/AIDS worldwide. In 2001, 5 million people were newly infected with HIV, and there were 3 million adult and child deaths due to HIV/AIDS. A full third of those people living with AIDS are aged 15-24. (World Health Organization, 2001). HIV/AIDS treatments exist, however, the drugs currently used in treatment modalities exhibit numerous side effects, require prolonged treatment that often induces drug resistance, and do
20 not result in complete eradication of the virus from the body.

The disease AIDS is the end result of an HIV-1 or HIV-2 virus following its own complex life cycle. The virion life cycle begins with the virion attaching itself to the host human T-4 lymphocyte immune cell through the bonding of a glycoprotein on the surface of the virion's protective coat with the CD4 glycoprotein on the lymphocyte cell.
25 Once attached, the virion sheds its glycoprotein coat, penetrates into the membrane of the host cell, and uncoats its RNA. The virion enzyme, reverse transcriptase, directs the process of transcribing the RNA into single-stranded DNA. The viral RNA is degraded and a second DNA strand is created. The now double-stranded DNA is integrated into the human cell's genes and those genes are used for virus reproduction.

At this point, RNA polymerase transcribes the integrated DNA into viral RNA. The viral RNA is translated into the precursor gag-pol fusion polyprotein. The polyprotein is then cleaved by the HIV protease to yield the mature viral proteins. Thus, HIV protease is responsible for regulating a cascade of cleavage events that lead to the virus particle's maturing into a virus that is capable of full infectivity.

The typical human immune system response, killing the invading virion, is taxed because the virus infects and kills the immune system's T cells. In addition, viral reverse transcriptase, the enzyme used in making a new virion particle, is not very specific, and causes transcription mistakes that result in continually changed glycoproteins on the surface of the viral protective coat. This lack of specificity decreases the immune system's effectiveness because antibodies specifically produced against one glycoprotein may be useless against another, hence reducing the number of antibodies available to fight the virus. The virus continues to reproduce while the immune response system continues to weaken. Eventually, the HIV largely holds free reign over the body's immune system, allowing opportunistic infections to set in and, without the administration of antiviral agents, immunomodulators, or both, death may result.

There are at least three critical points in the virus's life cycle which have been identified as possible targets for antiviral drugs: (1) the initial attachment of the virion to the T-4 lymphocyte or macrophage site; (2) the transcription of viral RNA to viral DNA (reverse transcriptase, RT); and (3) the processing of gag-pol protein by HIV protease.

Inhibition of the virus at the second critical point, the viral RNA to viral DNA transcription process, has provided a number of the current therapies used in treating AIDS. This transcription must occur for the virion to reproduce because the virion's genes are encoded in RNA and the host cell reads only DNA. By introducing drugs that block the reverse transcriptase from completing the formation of viral DNA, HIV-1, replication can be stopped.

A number of compounds that interfere with viral replication have been developed to treat AIDS. For example, nucleoside analogs, such as 3'-azido-3'-deoxythymidine (AZT), 2',3'-dideoxycytidine (ddC), 2',3'-dideoxythymidine (d4T), 2',3'-dideoxyinosine (ddI), and 2',3'-dideoxy-3'-thia-cytidine (3TC) have been shown to be relatively effective in halting HIV replication at the reverse transcriptase (RT) stage.

Even with the current success of reverse transcriptase inhibitors, it has been found that HIV patients can become resistant to a single inhibitor. Thus, it is desirable to

develop additional inhibitors to further combat HIV infection and inhibit the replication of drug resistant strains of HIV.

SUMMARY OF THE INVENTION

It has now been discovered that quinolones having novel structures effectively
5 inhibit the replication of HIV, including drug resistant strains of the virus. Selected quinolones of the invention are potent reverse transcriptase inhibitors. Accordingly, the present invention provides pharmaceutical compositions, and prophylactic and therapeutic treatments, diagnostic and prognostic methods and kits, and pharmaceutical screening methods that take advantage of the anti-HIV activity of the quinolones.

10 Because the quinolones of the invention inhibit HIV replication, the prophylactic or therapeutic administration of the quinolones is a treatment for HIV infection. Prophylactic treatments are especially useful for persons at high risk of HIV infection. Thus, the present invention provides methods of inhibiting HIV replication in a person by administering to the person a pharmaceutically effective amount of a quinolone. This
15 invention also provides pharmaceutical compositions comprising one or more quinolones in a pharmaceutically acceptable carrier. The compounds of the invention can be administered orally, parentally (including subcutaneous injection, intravenous, intramuscular, intrasternal or infusion techniques), by inhalation spray, topically, by absorption through a mucous membrane, or rectally, in dosage unit formulations containing conventional non-toxic
20 pharmaceutically acceptable carriers, adjuvants or vehicles.

Methods of inhibiting HIV replication described above can be applied to cells being cultured *in vitro*, as well.

In another aspect, the present invention provides a composition including at least one quinolone and a second therapeutic agent or agents. In an exemplary embodiment,
25 the second therapeutic agent is used to prevent or treat HIV infection. In another embodiment, the second therapeutic agent is used to treat an opportunistic infection associated with HIV infection. The second therapeutic is, for example, a protease inhibitor, a non-nucleoside reverse transcriptase inhibitor, a nucleoside reverse transcriptase inhibitor, an antiretroviral nucleoside, an entry inhibitor, or any other anti-viral agent effective to inhibit or
30 treat HIV infection. In another embodiment, the second therapeutic agent is selected from the group consisting of zidovudine, didanosine, stavudine, interferon, lamivudine, adefovir, nevirapine, delaviridine, loviride, saquinavir, indinavir, and AZT. In another embodiment,

the second therapeutic agent is an antibiotic or acyclovir. In still a further embodiment, the second agent is selected from immunomodulators, and entry inhibitors.

In another aspect, the present invention provides methods of treating or preventing HIV infection in a human comprising administering a quinolone of the invention to a subject. As discussed above, the quinolone is optionally combined with one or more additional therapeutic agents.

The invention also provides quinolones that are of use for inhibiting the replication of drug resistant, including multi-drug resistant, HIV mutants. The compounds of the invention have low cytotoxicity and display high potency against HIV and drug resistant strains of HIV. The compounds have been shown to inhibit replication of clinically observed drug resistant strains of HIV.

In another aspect, the present invention provides methods of inhibiting HIV infection in a CD4⁺ culture comprising the step of contacting the cell with a quinolone of the invention, either alone or in combination with a second therapeutic agent or a combination of other therapeutic agents. In one embodiment, the therapeutic agent or agents are used to treat or prevent HIV infection.

Other aspects, objects and advantages of the invention will be apparent from the detailed description that follows.

BRIEF DESCRIPTION OF THE DRAWINGS

FIG. 1 is a table displaying exemplary compounds of the invention.

DETAILED DESCRIPTION OF THE INVENTION AND PREFERRED EMBODIMENTS

The present invention provides new compositions and methods for preventing or ameliorating viral, *e.g.*, HIV infection, killing virally infected cells, *e.g.*, HIV infected cells and generally, inhibiting viral, preferably HIV, replication. The present invention is, in part, based on the surprising discovery that the quinolones of the invention effectively inhibit HIV infection, kill HIV infected cells and/or prevent HIV infection in the individual. Moreover, the compounds of the invention inhibit the replication of drug resistant strains of HIV.

The present invention provides compounds and pharmaceutical compositions that include those compounds. Moreover, the invention also provides methods of inhibiting HIV infection or replication by administering at least one compound of the invention to a patient in need of such treatment.

Definitions

“Reactive functional group,” as used herein refers to groups including, but not limited to, olefins, acetylenes, alcohols, phenols, ethers, oxides, halides, aldehydes, ketones, carboxylic acids, esters, amides, cyanates, isocyanates, thiocyanates, isothiocyanates, amines, hydrazines, hydrazones, hydrazides, diazo, diazonium, nitro, nitriles, mercaptans, sulfides, disulfides, sulfoxides, sulfones, sulfonic acids, sulfinic acids, acetals, ketals, anhydrides, sulfates, sulfenic acids isonitriles, amidines, imides, imidates, nitrones, hydroxylamines, oximes, hydroxamic acids thiohydroxamic acids, allenes, ortho esters, sulfites, enamines, ynamines, ureas, pseudoureas, semicarbazides, carbodiimides, carbamates, imines, azides, azo compounds, azoxy compounds, and nitroso compounds. Reactive functional groups also include those used to prepare bioconjugates, e.g., N-hydroxysuccinimide esters, maleimides and the like. Methods to prepare each of these functional groups are well known in the art and their application to or modification for a particular purpose is within the ability of one of skill in the art (*see*, for example, Sandler and Karo, eds. ORGANIC FUNCTIONAL GROUP PREPARATIONS, Academic Press, San Diego, 1989).

“Non-covalent protein binding groups” are moieties that interact with an intact or denatured polypeptide in an associative manner. The interaction may be either reversible or irreversible in a biological milieu. The incorporation of a “non-covalent protein binding group” into a quinolone of the invention provides the compound with the ability to interact with a polypeptide in a non-covalent manner. Exemplary non-covalent interactions include hydrophobic-hydrophobic and electrostatic interactions. Exemplary “non-covalent protein binding groups” include anionic groups, e.g., phosphate, thiophosphate, phosphonate, carboxylate, boronate, sulfate, sulfone, thiosulfate, and thiosulfonate.

As used herein, “linking member” refers to a covalent chemical bond that includes at least one heteroatom. Exemplary linking members include $-C(O)NH-$, $-C(O)O-$, $-NH-$, $-S-$, $-O-$, and the like.

The term “targeting group” is intended to mean a moiety that is: (1) able to actively direct the entity to which it is attached (e.g., a quinolone) to a target region, e.g., an HIV infected cell; or (2) is preferentially passively absorbed by or entrained within a target tissue. The targeting group can be a small molecule, which is intended to include both non-peptides and peptides. The targeting group can also be a macromolecule, which includes, but is not limited to, saccharides, lectins, receptors, ligand for receptors, proteins such as BSA, antibodies, poly(ethers), dendrimers, poly(amino acids) and so forth.

The term "cleavable group" is intended to mean a moiety that allows for release of a quinolone from a conjugate by cleaving a bond linking the quinolone (or quinolone linker arm construct) to the remainder of the conjugate. Such cleavage is either chemical in nature, or enzymatically mediated. Exemplary enzymatically cleavable groups
5 include natural amino acids or peptide sequences that end with a natural amino acid.

In addition to enzymatically cleavable sites, it is within the scope of the present invention to include one or more sites that are cleaved by the action of an agent other than an enzyme. Exemplary non-enzymatic cleavage agents include, but are not limited to, acids, bases, light (e.g., nitrobenzyl derivatives, phenacyl groups, benzoin esters), and heat.

10 Many cleavable groups are known in the art. *See, for example, Jung et al., Biochem. Biophys. Acta*, **761**: 152-162 (1983); Joshi *et al., J. Biol. Chem.*, **265**: 14518-14525 (1990); Zarling *et al., J. Immunol.*, **124**: 913-920 (1980); Bouizar *et al., Eur. J. Biochem.*, **155**: 141-147 (1986); Park *et al., J. Biol. Chem.*, **261**: 205-210 (1986); Browning *et al., J. Immunol.*, **143**: 1859-1867 (1989). Moreover a broad range of cleavable, bifunctional (both homo- and
15 hetero-bifunctional) spacer arms are commercially available from suppliers such as Pierce.

The symbol ~~, whether utilized as a bond or displayed perpendicular to a bond indicates the point at which the displayed moiety is attached to the remainder of the molecule, solid support, etc.

Certain compounds of the present invention can exist in unsolvated forms as
20 well as solvated forms, including hydrated forms. In general, the solvated forms are equivalent to unsolvated forms and are encompassed within the scope of the present invention. Certain compounds of the present invention may exist in multiple crystalline or amorphous forms. In general, all physical forms are equivalent for the uses contemplated by the present invention and are intended to be within the scope of the present invention.

25 Certain compounds of the present invention possess asymmetric carbon atoms (optical centers) or double bonds; the racemates, diastereomers, geometric isomers and individual isomers are encompassed within the scope of the present invention.

The compounds of the invention may be prepared as a single isomer (e.g., enantiomer, cis-trans, positional, diastereomer) or as a mixture of isomers. In a preferred
30 embodiment, the compounds are prepared as substantially a single isomer. Methods of preparing substantially isomerically pure compounds are known in the art. For example, enantiomerically enriched mixtures and pure enantiomeric compounds can be prepared by using synthetic intermediates that are enantiomerically pure in combination with reactions that either leave the stereochemistry at a chiral center unchanged or result in its complete

inversion. Alternatively, the final product or intermediates along the synthetic route can be resolved into a single stereoisomer. Techniques for inverting or leaving unchanged a particular stereocenter, and those for resolving mixtures of stereoisomers are well known in the art and it is well within the ability of one of skill in the art to choose and appropriate method for a particular situation. See, generally, Furniss *et al.* (eds.), VOGEL'S
5 ENCYCLOPEDIA OF PRACTICAL ORGANIC CHEMISTRY 5TH ED., Longman Scientific and Technical Ltd., Essex, 1991, pp. 809-816; and Heller, *Acc. Chem. Res.* **23**: 128 (1990).

The compounds of the present invention may also contain unnatural proportions of atomic isotopes at one or more of the atoms that constitute such compounds.
10 For example, the compounds may be radiolabeled with radioactive isotopes, such as for example tritium (³H), iodine-125 (¹²⁵I) or carbon-14 (¹⁴C). All isotopic variations of the compounds of the present invention, whether radioactive or not, are intended to be encompassed within the scope of the present invention.

Where substituent groups are specified by their conventional chemical
15 formulae, written from left to right, they equally encompass the chemically identical substituents, which would result from writing the structure from right to left, e.g., -CH₂O- is intended to also recite -OCH₂-.

The term "alkyl," by itself or as part of another substituent means, unless otherwise stated, a straight or branched chain, or cyclic hydrocarbon radical, or combination
20 thereof, which may be fully saturated, mono- or polyunsaturated and can include di- and multivalent radicals, having the number of carbon atoms designated (*i.e.* C₁-C₁₀ means one to ten carbons). Examples of saturated hydrocarbon radicals include, but are not limited to, groups such as methyl, ethyl, n-propyl, isopropyl, n-butyl, t-butyl, isobutyl, sec-butyl, cyclohexyl, (cyclohexyl)methyl, cyclopropylmethyl, homologs and isomers of, for example,
25 n-pentyl, n-hexyl, n-heptyl, n-octyl, and the like. An unsaturated alkyl group is one having one or more double bonds or triple bonds. Examples of unsaturated alkyl groups include, but are not limited to, vinyl, 2-propenyl, crotyl, 2-isopentenyl, 2-(butadienyl), 2,4-pentadienyl, 3-(1,4-pentadienyl), ethynyl, 1- and 3-propynyl, 3-butylnyl, and the higher homologs and isomers. The term "alkyl," unless otherwise noted, is also meant to include those derivatives
30 of alkyl defined in more detail below, such as "heteroalkyl." Alkyl groups that are limited to hydrocarbon groups are termed "homoalkyl".

The term "alkylene" by itself or as part of another substituent means a divalent radical derived from an alkane, as exemplified, but not limited, by -CH₂CH₂CH₂CH₂-, and further includes those groups described below as "heteroalkylene." Typically, an alkyl (or

alkylene) group will have from 1 to 24 carbon atoms, with those groups having 10 or fewer carbon atoms being preferred in the present invention. A "lower alkyl" or "lower alkylene" is a shorter chain alkyl or alkylene group, generally having eight or fewer carbon atoms.

The terms "alkoxy," "alkylamino" and "alkylthio" (or thioalkoxy) are used in their conventional sense, and refer to those alkyl groups attached to the remainder of the molecule via an oxygen atom, an amino group, or a sulfur atom, respectively.

The term "heteroalkyl," by itself or in combination with another term, means, unless otherwise stated, a stable straight or branched chain, or cyclic hydrocarbon radical, or combinations thereof, consisting of the stated number of carbon atoms and at least one heteroatom selected from the group consisting of O, N, Si and S, and wherein the nitrogen and sulfur atoms may optionally be oxidized and the nitrogen heteroatom may optionally be quaternized. The heteroatom(s) O, N and S and Si may be placed at any interior position of the heteroalkyl group or at the position at which the alkyl group is attached to the remainder of the molecule. Examples include, but are not limited to, $-\text{CH}_2-\text{CH}_2-\text{O}-\text{CH}_3$, $-\text{CH}_2-\text{CH}_2-\text{NH}-\text{CH}_3$, $-\text{CH}_2-\text{CH}_2-\text{N}(\text{CH}_3)-\text{CH}_3$, $-\text{CH}_2-\text{S}-\text{CH}_2-\text{CH}_3$, $-\text{CH}_2-\text{CH}_2-\text{S}(\text{O})-\text{CH}_3$, $-\text{CH}_2-\text{CH}_2-\text{S}(\text{O})_2-\text{CH}_3$, $-\text{CH}=\text{CH}-\text{O}-\text{CH}_3$, $-\text{Si}(\text{CH}_3)_3$, $-\text{CH}_2-\text{CH}=\text{N}-\text{OCH}_3$, and $-\text{CH}=\text{CH}-\text{N}(\text{CH}_3)-\text{CH}_3$. Up to two heteroatoms may be consecutive, such as, for example, $-\text{CH}_2-\text{NH}-\text{OCH}_3$ and $-\text{CH}_2-\text{O}-\text{Si}(\text{CH}_3)_3$. Similarly, the term "heteroalkylene" by itself or as part of another substituent means a divalent radical derived from heteroalkyl, as exemplified, but not limited by, $-\text{CH}_2-\text{CH}_2-\text{S}-\text{CH}_2-\text{CH}_2-$ and $-\text{CH}_2-\text{S}-\text{CH}_2-\text{CH}_2-\text{NH}-\text{CH}_2-$. For heteroalkylene groups, heteroatoms can also occupy either or both of the chain termini (*e.g.*, alkyleneoxy, alkylenedioxy, alkyleneamino, alkylenediamino, and the like). Still further, for alkylene and heteroalkylene linking groups, no orientation of the linking group is implied by the direction in which the formula of the linking group is written. For example, the formula $-\text{C}(\text{O})_2\text{R}'$ represents both $-\text{C}(\text{O})_2\text{R}'$ and $-\text{R}'\text{C}(\text{O})_2-$.

The terms "cycloalkyl" and "heterocycloalkyl", by themselves or in combination with other terms, represent, unless otherwise stated, cyclic versions of "alkyl" and "heteroalkyl", respectively. Additionally, for heterocycloalkyl, a heteroatom can occupy the position at which the heterocycle is attached to the remainder of the molecule. Examples of cycloalkyl include, but are not limited to, cyclopentyl, cyclohexyl, 1-cyclohexenyl, 3-cyclohexenyl, cycloheptyl, and the like. Examples of heterocycloalkyl include, but are not limited to, 1-(1,2,5,6-tetrahydropyridyl), 1-piperidiny, 2-piperidiny, 3-piperidiny, 4-morpholinyl, 3-morpholinyl, tetrahydrofuran-2-yl, tetrahydrofuran-3-yl, tetrahydrothien-2-yl, tetrahydrothien-3-yl, 1-piperazinyl, 2-piperazinyl, and the like.

The terms “halo” or “halogen,” by themselves or as part of another substituent, mean, unless otherwise stated, a fluorine, chlorine, bromine, or iodine atom. Additionally, terms such as “haloalkyl,” are meant to include monohaloalkyl and polyhaloalkyl. For example, the term “halo(C₁-C₄)alkyl” is meant to include, but not be limited to,

5 trifluoromethyl, 2,2,2-trifluoroethyl, 4-chlorobutyl, 3-bromopropyl, and the like.

The term “aryl” means, unless otherwise stated, a polyunsaturated, aromatic, substituent that can be a single ring or multiple rings (preferably from 1 to 3 rings), which are fused together or linked covalently. The term “heteroaryl” refers to aryl groups (or rings) that contain from one to four heteroatoms selected from N, O, and S, wherein the nitrogen and sulfur atoms are optionally oxidized, and the nitrogen atom(s) are optionally quaternized. A

10 heteroaryl group can be attached to the remainder of the molecule through a heteroatom. Non-limiting examples of aryl and heteroaryl groups include phenyl, 1-naphthyl, 2-naphthyl, 4-biphenyl, 1-pyrrolyl, 2-pyrrolyl, 3-pyrrolyl, 3-pyrazolyl, 2-imidazolyl, 4-imidazolyl, pyrazinyl, 2-oxazolyl, 4-oxazolyl, 2-phenyl-4-oxazolyl, 5-oxazolyl, 3-isoxazolyl, 4-
15 isoxazolyl, 5-isoxazolyl, 2-thiazolyl, 4-thiazolyl, 5-thiazolyl, 2-furyl, 3-furyl, 2-thienyl, 3-thienyl, 2-pyridyl, 3-pyridyl, 4-pyridyl, 2-pyrimidyl, 4-pyrimidyl, 5-benzothiazolyl, purinyl, 2-benzimidazolyl, 5-indolyl, 1-isoquinolyl, 5-isoquinolyl, 2-quinoxaliny, 5-quinoxaliny, 3-quinolyl, tetrazolyl, benzo[b]furanyl, benzo[b]thienyl, 2,3-dihydrobenzo[1,4]dioxin-6-yl, benzo[1,3]dioxol-5-yl and 6-quinolyl. Substituents for each of the above noted aryl and
20 heteroaryl ring systems are selected from the group of acceptable substituents described below.

For brevity, the term “aryl” when used in combination with other terms (*e.g.*, aryloxy, arylthioxy, arylalkyl) includes both aryl and heteroaryl rings as defined above. Thus, the term “arylalkyl” is meant to include those radicals in which an aryl group is
25 attached to an alkyl group (*e.g.*, benzyl, phenethyl, pyridylmethyl and the like) including those alkyl groups in which a carbon atom (*e.g.*, a methylene group) has been replaced by, for example, an oxygen atom (*e.g.*, phenoxymethyl, 2-pyridyloxymethyl, 3-(1-naphthyloxy)propyl, and the like).

Each of the above terms (*e.g.*, “alkyl,” “heteroalkyl,” “aryl” and “heteroaryl”) is meant to include both substituted and unsubstituted forms of the indicated radical.
30 Preferred substituents for each type of radical are provided below.

Substituents for the alkyl and heteroalkyl radicals (including those groups often referred to as alkylene, alkenyl, heteroalkylene, heteroalkenyl, alkynyl, cycloalkyl, heterocycloalkyl, cycloalkenyl, and heterocycloalkenyl) are generically referred to as “alkyl

group substituents," and they can be one or more of a variety of groups selected from, but not limited to: -OR', =O, =NR', =N-OR', -NR'R'', -SR', -halogen, -SiR'R''R''', -OC(O)R', -C(O)R', -CO₂R', -CONR'R'', -OC(O)NR'R'', -NR''C(O)R', -NR'-C(O)NR''R''', -NR''C(O)₂R', -NR-C(NR'R''R''')=NR''', -NR-C(NR'R'')=NR''', -S(O)R', -S(O)₂R', -

- 5 S(O)₂NR'R'', -NRSO₂R', -CN and -NO₂ in a number ranging from zero to (2m'+1), where m' is the total number of carbon atoms in such radical. R', R'', R''' and R'''' each preferably independently refer to hydrogen, substituted or unsubstituted heteroalkyl, substituted or unsubstituted aryl, e.g., aryl substituted with 1-3 halogens, substituted or unsubstituted alkyl, alkoxy or thioalkoxy groups, or arylalkyl groups. When a compound of the invention
- 10 includes more than one R group, for example, each of the R groups is independently selected as are each R', R'', R''' and R'''' groups when more than one of these groups is present. When R' and R'' are attached to the same nitrogen atom, they can be combined with the nitrogen atom to form a 5-, 6-, or 7-membered ring. For example, -NR'R'' is meant to include, but not be limited to, 1-pyrrolidinyl and 4-morpholinyl. From the above discussion of substituents,
- 15 one of skill in the art will understand that the term "alkyl" is meant to include groups including carbon atoms bound to groups other than hydrogen groups, such as haloalkyl (e.g., -CF₃ and -CH₂CF₃) and acyl (e.g., -C(O)CH₃, -C(O)CF₃, -C(O)CH₂OCH₃, and the like).

- Similar to the substituents described for the alkyl radical, substituents for the aryl and heteroaryl groups are generically referred to as "aryl group substituents." The
- 20 substituents are selected from, for example: halogen, -OR', =O, =NR', =N-OR', -NR'R'', -SR', -halogen, -SiR'R''R''', -OC(O)R', -C(O)R', -CO₂R', -CONR'R'', -OC(O)NR'R'', -NR''C(O)R', -NR'-C(O)NR''R''', -NR''C(O)₂R', -NR-C(NR'R''R''')=NR''', -NR-C(NR'R'')=NR''', -S(O)R', -S(O)₂R', -S(O)₂NR'R'', -NRSO₂R', -CN and -NO₂, -R', -N₃, -CH(Ph)₂, fluoro(C₁-C₄)alkoxy, and fluoro(C₁-C₄)alkyl, in a number ranging from zero to
- 25 the total number of open valences on the aromatic ring system; and where R', R'', R''' and R'''' are preferably independently selected from hydrogen, substituted or unsubstituted alkyl, substituted or unsubstituted heteroalkyl, substituted or unsubstituted aryl and substituted or unsubstituted heteroaryl. When a compound of the invention includes more than one R group, for example, each of the R groups is independently selected as are each R', R'', R'''
- 30 and R'''' groups when more than one of these groups is present. In the schemes that follow, the symbol X represents "R" as described above.

Two of the substituents on adjacent atoms of the aryl or heteroaryl ring may optionally be replaced with a substituent of the formula -T-C(O)-(CRR')_q-U-, wherein T and U are independently -NR-, -O-, -CRR'- or a single bond, and q is an integer of from 0 to 3.

Alternatively, two of the substituents on adjacent atoms of the aryl or heteroaryl ring may optionally be replaced with a substituent of the formula $-A-(CH_2)_r-B-$, wherein A and B are independently $-CRR'-$, $-O-$, $-NR-$, $-S-$, $-S(O)-$, $-S(O)_2-$, $-S(O)_2NR'-$ or a single bond, and r is an integer of from 1 to 4. One of the single bonds of the new ring so formed may optionally
5 be replaced with a double bond. Alternatively, two of the substituents on adjacent atoms of the aryl or heteroaryl ring may optionally be replaced with a substituent of the formula $-(CRR')_s-X-(CR''R''')_d-$, where s and d are independently integers of from 0 to 3, and X is $-O-$, $-NR'-$, $-S-$, $-S(O)-$, $-S(O)_2-$, or $-S(O)_2NR'-$. The substituents R, R', R'' and R''' are preferably independently selected from hydrogen or substituted or unsubstituted (C_1-C_6) alkyl.

10 As used herein, the term "heteroatom" is meant to include oxygen (O), nitrogen (N), sulfur (S) and silicon (Si).

"Protecting group," as used herein refers to a portion of a substrate that is substantially stable under a particular reaction condition, but which is cleaved from the substrate under a different reaction condition. A protecting group can also be selected such
15 that it participates in the direct oxidation of the aromatic ring component of the compounds of the invention. For examples of useful protecting groups, *see*, for example, Greene *et al.*, PROTECTIVE GROUPS IN ORGANIC SYNTHESIS, John Wiley & Sons, New York, 1991.

As used herein, "pharmaceutically acceptable salt" refers to a salt that retains the desired biological activity of the parent compound and does not impart any undesired
20 toxicological effects. Examples of such salts include, but are not limited to, (a) acid addition salts formed with inorganic acids, for example hydrochloric acid, hydrobromic acid, sulfuric acid, phosphoric acid, nitric acid and the like; and salts formed with organic acids such as, for example, acetic acid, oxalic acid, tartaric acid, succinic acid, maleic acid, fumaric acid, gluconic acid, citric acid, malic acid, ascorbic acid, benzoic acid, tannic acid, pantoic acid,
25 alginic acid, polyglutamic acid, naphthalenesulfonic acids, naphthalenedisulfonic acids, polygalacturonic acid; (b) salts with polyvalent metal cations such as zinc, calcium, bismuth, barium, magnesium, aluminum, copper, cobalt, nickel, cadmium, and the like; or (c) salts formed with an organic cation formed from N,N'-dibenzylethylenediamine or ethylenediamine; or (d) combinations of (a) and (b) or (c), e.g., a zinc tannate salt; and the
30 like. The preferred acid addition salts are the trifluoroacetate salt and the acetate salt.

A "disorder associated with HIV infection" or "disease associated with HIV infection" refers to a disease state which is marked by HIV infection. Such disorders associated with HIV infection include, but are not limited to, AIDS, Kaposi's sarcoma, opportunistic infections such as those caused by *Pneumocystis carinii* and *Mycobacterium*

tuberculosis; oral lesions, including thrush, hairy leukoplakia, and aphthous ulcers; generalized lymphadenopathy, shingles, thrombocytopenia, aseptic meningitis, and neurologic disease such as toxoplasmosis, cryptococcosis, CMV infection, primary CNS lymphoma, and HIV-associated dementia, peripheral neuropathies, seizures, and myopathy.

5 As used herein, "reverse transcriptase (RT) activity" means the ability to effect reverse transcription of retroviral RNA to proviral DNA. One means by which RT activity can be determined is by measuring viral replication. One measure of HIV-1 viral replication is the p24 core antigen enzyme immunoassay, for example, using the assay commercially available from Coulter Corporation/Immunotech, Inc. (Westbrooke, Mich.). Another means
10 by which RT activity is analyzed is by assay of recombinant HIV-1 reverse transcriptase (rRT) activity, for example, using the Quan-T-RT assay system commercially available from Amersham (Arlington Heights, Ill.) and described in Bosworth, et al., *Nature*, 1989, 341:167-168.

As used herein, "HIV reverse transcriptase inhibitor" is intended to refer to
15 both nucleoside and non-nucleoside inhibitors of HIV reverse transcriptase (RT). Examples of nucleoside RT inhibitors include, but are not limited to, AZT, ddC, ddI, d4T, and 3TC. Examples of non-nucleoside RT inhibitors include, but are no limited to, delavirdine (Pharmacia and Upjohn U90152S), efavirenz (DuPont), nevirapine (Boehringer Ingelheim), Ro 18,893 (Roche), trovirdine (Lilly), MKC-442 (Triangle), HBY 097 (Hoechst), ACT
20 (Korean Research Institute), UC-781 (Rega Institute), UC-782 (Rega Institute), RD4-2025 (Tosoh Co. Ltd.), and MEN 10979 (Menarini Farmaceutici).

As used herein, "HIV protease inhibitor" is intended to refer to compounds that inhibit HIV protease. Examples include, but are not limited, saquinavir (Roche, Ro31-8959), ritonavir (Abbott, ABT-538), indinavir (Merck, MK-639), amprenavir (Vertex/Glaxo
25 Wellcome), nelfinavir (Agouron, AG-1343), palinavir (Boehringer Ingelheim), BMS-232623 (Bristol-Myers Squibb), GS3333 (Gilead Sciences), KNI-413 (Japan Energy), KNI-272 (Japan Energy), LG-71350 (LG Chemical), CGP-61755 (Ciba-Geigy), PD 173606 (Parke Davis), PD 177298 (Parke Davis), PD 178390 (Parke Davis), PD 178392 (Parke Davis), U-140690 (Pharmacia and Upjohn), and ABT-378. Additional examples include the cyclic
30 protease inhibitors disclosed in WO93/07128, WO 94/19329, WO 94/22840, and PCT Application Number US96/03426.

As used herein, a compound that "inhibits replication of human immunodeficiency virus (HIV)" means a compound that, when contacted with HIV, for example, via HIV-infected cells, effects a reduction in the amount of HIV as compared with

untreated control. Inhibition of replication of HIV can be measured by various means known in the art, for example, the p24 assay.

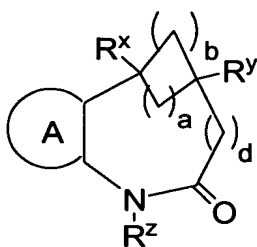
By "therapeutically effective dose" herein is meant a dose that produces effects for which it is administered. The exact dose will depend on the purpose of the treatment, and is ascertainable by one skilled in the art using known techniques (*see, e.g.,* Lieberman, *Pharmaceutical Dosage Forms* (vols. 1-3, 1992); Lloyd, *The Art, Science and Technology of Pharmaceutical Compounding* (1999); and Pickar, *Dosage Calculations* (1999)).

"Mutant HIV" means a strain of HIV having one or more mutated or altered amino acids as compared with wild type.

"Multi-Drug Resistant HIV" means one or more HIV strain that is resistant to treatment with one or more chemotherapeutic agent.

Compounds

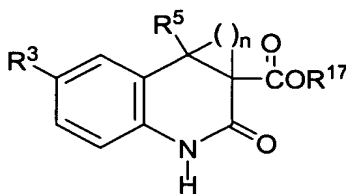
In a first aspect, the invention provides a compound according to Formula I:



in which the symbol A represents a ring system that is selected from substituted or unsubstituted aryl and substituted or unsubstituted heteroaryl. The symbols a and d represent integers that are independently selected from 0, 1 and 2, and the symbol b is selected from the integers 1 and 2.

The symbols R^x , R^y and R^z represent members independently selected from H, substituted or unsubstituted alkyl, substituted or unsubstituted cycloalkyl, substituted or unsubstituted heteroalkyl, substituted or unsubstituted aryl, substituted or unsubstituted heteroaryl, or another "alkyl substituent" as defined hereinabove. Further detail regarding the identities of the "R" groups set forth above, is provided in the succeeding paragraphs.

In an exemplary embodiment, the invention provides compounds having the formula:

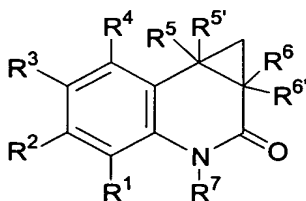


(II)

The index "n" represents an integer from 1 to 4, preferably 1 to 2. The identities of R¹⁷, R⁵ and R³ are discussed further below.

In an exemplary embodiment, the invention provides compounds according to

5 Formula II:



(II).

In Formula II, the symbols R¹, R², R³ and R⁴ independently represent H, substituted or unsubstituted alkyl, substituted or unsubstituted cycloalkyl, substituted or unsubstituted heteroalkyl, substituted or unsubstituted aryl, substituted or unsubstituted heteroaryl, OR⁸, NO₂, CN or halogen. R⁸ is H or substituted or unsubstituted alkyl.

The symbols R⁵ and R^{5'} independently represent H, substituted or unsubstituted alkyl (e.g., unsaturated alkyl), substituted or unsubstituted cycloalkyl, substituted or unsubstituted heteroalkyl, substituted or unsubstituted heterocycloalkyl, substituted or unsubstituted aryl, substituted or unsubstituted heteroaryl, CN or C(O)R⁹. In an exemplary embodiment, R⁵ is selected from substituted or unsubstituted C₁-C₆ alkyl and substituted or unsubstituted C₁-C₆ cycloalkyl.

The symbol R⁹ represents H, substituted or unsubstituted alkyl, substituted or unsubstituted cycloalkyl, substituted or unsubstituted heteroalkyl, NR¹⁰R¹¹ or OR¹¹. R¹⁰ is selected from H, substituted or unsubstituted alkyl and OR¹². The symbol R¹² represents H, substituted or unsubstituted alkyl, substituted or unsubstituted cycloalkyl and substituted or unsubstituted heteroalkyl. R¹¹ is selected from H, C(O)R¹³, substituted or unsubstituted alkyl, substituted or unsubstituted cycloalkyl, substituted or unsubstituted heteroalkyl, substituted or unsubstituted heterocycloalkyl and substituted or unsubstituted aryl. In an exemplary embodiment, R¹¹ is substituted or unsubstituted benzyl.

The groups represented by R¹⁰ and R¹¹, together with the nitrogen to which they are bound, are optionally joined to form a substituted or unsubstituted heterocycloalkyl

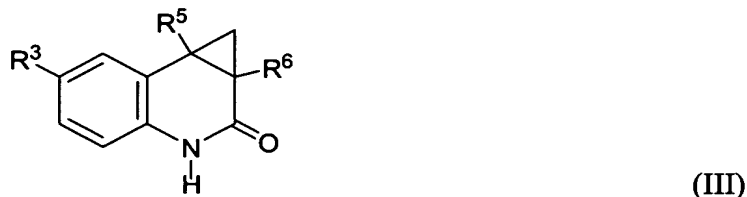
ring system having from 3 to 7 members. In an exemplary embodiment, the heterocycloalkyl ring is substituted with substituted or unsubstituted alkyl.

The symbol R^{13} represents H, substituted or unsubstituted alkyl, substituted or unsubstituted cycloalkyl, substituted or unsubstituted heteroalkyl or $NR^{14}R^{15}$, and R^{14} and R^{15} are members independently selected from H, substituted or unsubstituted alkyl, substituted or unsubstituted cycloalkyl, and substituted or unsubstituted heteroalkyl.

In an exemplary embodiment, at least one of R^{10} and R^{11} is substituted or unsubstituted C_1 - C_6 alkyl.

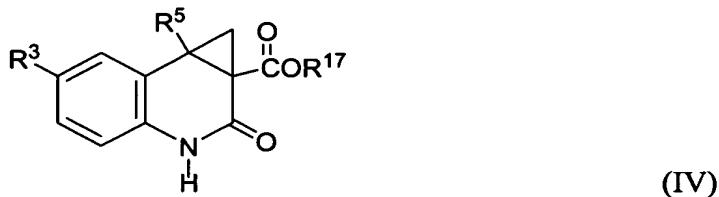
The symbols R^6 and $R^{6'}$ represent H, substituted or unsubstituted alkyl, substituted or unsubstituted cycloalkyl, substituted or unsubstituted aryl, substituted or unsubstituted heteroaryl, or $C(O)R^{16}$, in which R^{16} is substituted or unsubstituted alkyl, substituted or unsubstituted cycloalkyl, substituted or unsubstituted heteroalkyl, $NR^{17}R^{18}$ or OR^{17} . R^{17} and R^{18} are independently selected from H, substituted or unsubstituted alkyl, substituted or unsubstituted cycloalkyl, substituted or unsubstituted heteroalkyl and substituted or unsubstituted aryl.

In another exemplary embodiment, the invention provides compounds according to Formula III:



in which the identity of R^3 , R^5 and R^6 are substantially as described above.

In yet a further exemplary embodiment, the invention provides compound according to Formula IV:

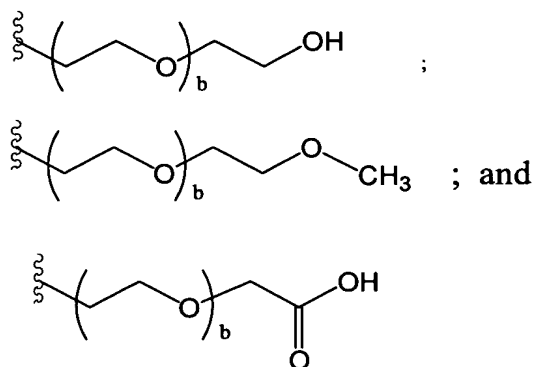


in which the identity of R^3 , R^5 and R^6 are substantially as described above.

In another exemplary embodiment, at least one of R^1 - R^7 is a moiety that increases the water-solubility of the parent compound. Exemplary moieties of use for increasing a compound's water solubility include ethers and polyethers, e.g., a member selected from ethylene glycol, and ethylene glycol oligomers, having a molecular weight of

from about 60 daltons to about 10,000 daltons, and more preferably of from about 100 daltons to about 1,000 daltons.

Representative polyether-based substituents include, but are not limited to, the following structures:



in which b is preferably a number from 1 to 100, inclusive. Other functionalized polyethers are known to those of skill in the art, and many are commercially available from, for example, Shearwater Polymers, Inc. (Alabama).

10 In another exemplary embodiment, at least one of R^1 - R^7 is a linker moiety that includes a reactive functional group for conjugating the compound to another molecule or to a surface. The linkers of use in the compounds of the invention can also include a cleaveable group. In an exemplary embodiment, the cleaveable group is interposed between the quinolone core and a targeting agent or macromolecular backbone. Representative useful reactive groups are discussed in greater detail in succeeding sections. Additional information
15 on useful reactive groups is known to those of skill in the art. *See*, for example, Hermanson, BIOCONJUGATE TECHNIQUES, Academic Press, San Diego, 1996.

Reactive Functional Groups

20 As discussed above, the quinolone core of the compounds of the invention are optionally tethered to other species by means of bonds formed between a reactive functional group on the quinolone or a linker attached to the quinolone, and a reactive functional group of complementary reactivity on the other species. For clarity of illustration the succeeding discussion focuses on the conjugation of representative quinolones of the invention to polymers, including poly(ethers) and dendrimers, and to targeting agents useful for translocating the quinolone-targeting agent conjugate across a membrane. The focus
25 exemplifies selected embodiments of the invention from which others are readily inferred by

one of skill in the art. No limitation of the invention is implied, by focusing the discussion on the representative embodiments.

Exemplary quinolones of the invention bear a reactive functional group, which is generally located on the quinolone ring or on a substituted or unsubstituted alkyl or heteroalkyl chain attached to the ring, allowing their facile attachment to another species. A convenient location for the reactive group is the terminal position of an alkyl or heteroalkyl substituent of the quinolone core.

Reactive groups and classes of reactions useful in practicing the present invention are generally those that are well known in the art of bioconjugate chemistry.

Currently favored classes of reactions available with reactive analogues are those proceeding under relatively mild conditions. These include, but are not limited to nucleophilic substitutions (*e.g.*, reactions of amines and alcohols with acyl halides, active esters), electrophilic substitutions (*e.g.*, enamine reactions) and additions to carbon-carbon and carbon-heteroatom multiple bonds (*e.g.*, Michael reaction, Diels-Alder addition). These and other useful reactions are discussed in, for example, March, ADVANCED ORGANIC CHEMISTRY, 3rd Ed., John Wiley & Sons, New York, 1985; Hermanson, BIOCONJUGATE TECHNIQUES, Academic Press, San Diego, 1996; and Feeney *et al.*, MODIFICATION OF PROTEINS; Advances in Chemistry Series, Vol. 198, American Chemical Society, Washington, D.C., 1982.

Exemplary reaction types include the reaction of carboxyl groups and various derivatives thereof including, but not limited to, N-hydroxysuccinimide esters, N-hydroxybenzotriazole esters, acid halides, acyl imidazoles, thioesters, p-nitrophenyl esters, alkyl, alkenyl, alkynyl and aromatic esters. Hydroxyl groups can be converted to esters, ethers, aldehydes, *etc.* Haloalkyl groups are converted to new species by reaction with, for example, an amine, a carboxylate anion, thiol anion, carbanion, or an alkoxide ion. Dienophile (*e.g.*, maleimide) groups participate in Diels-Alder. Aldehyde or ketone groups can be converted to imines, hydrazones, semicarbazones or oximes, or reacted via such mechanisms as Grignard addition or alkyllithium addition. Sulfonyl halides react readily with amines, for example, to form sulfonamides. Amine or sulfhydryl groups are, for example, acylated, alkylated or oxidized. Alkenes can be converted to an array of new species using cycloadditions, acylation, Michael addition, *etc.* Epoxides react readily with amines and hydroxyl compounds.

Exemplary combinations of reactive functional groups found on a ligand of the invention and on a targeting moiety (or polymer or linker) are set forth in Table 1.

TABLE 1

	Chemical Functionality 1	Chemical Functionality 2	Linkage
5	Hydroxy	Carboxy	Ester
		Hydroxy	Carbonate
		Amine	Carbamate
10		SO ₃	Sulfate
		PO ₃	Phosphate
		Carboxy	Acyloxyalkyl
		Ketone	Ketal
		Aldehyde	Acetal
15		Hydroxy	Anhydride
		Mercapto	Disulfide
	Mercapto	Carboxy	Acyloxyalkyl
			Thioether
		Carboxy	Thioester
20		Carboxy	Amino amide
		Mercapto	Thioester
		Carboxy	Acyloxyalkyl ester
		Carboxy	Acyloxyalkyl amide
25		Amino	Acyloxyalkoxy carbonyl
		Carboxy	Anhydride
		Carboxy	N-acylamide
30		Hydroxy	Ester
		Hydroxy	Hydroxymethyl ketone ester
		Hydroxy	Alkoxycarbonyl oxyalkyl
35		Carboxy	Acyloxyalkylamine
		Carboxy	Acyloxyalkylamide
		Amino	Urea
		Carboxy	Amide
40		Carboxy	Acyloxyalkoxycarbonyl
		Amide	N-Mannich base
		Carboxy	Acyloxyalkyl carbamate
	Phosphate oxygen ester	Hydroxy	Phosphate
		Amine	Phosphoramidate
		Mercapto	Thiophosphate ester
45	Ketone Sulfonamide	Carboxy	Enol ester
		Carboxy	Acyloxyalkyl sulfonamide
		Ester	N-sulfonyl- imidate

50 One skilled in the art will readily appreciate that many of these linkages may
be produced in a variety of ways and using a variety of conditions. For the preparation of
esters, *see, e.g.*, March *supra* at 1157; for thioesters, *see*, March, *supra* at 362-363, 491, 720-
722, 829, 941, and 1172; for carbonates, *see*, March, *supra* at 346-347; for carbamates, *see*,
March, *supra* at 1156-57; for amides, *see*, March *supra* at 1152; for ureas and thioureas, *see*,
55 March *supra* at 1174; for acetals and ketals, *see*, Greene *et al. supra* 178-210 and March

supra at 1146; for acyloxyalkyl derivatives, *see*, PRODRUGS: TOPICAL AND OCULAR DRUG DELIVERY, K. B. Sloan, ed., Marcel Dekker, Inc., New York, 1992; for enol esters, *see*, March *supra* at 1160; for N-sulfonylimidates, *see*, Bundgaard *et al.*, *J. Med. Chem.*, **31**:2066 (1988); for anhydrides, *see*, March *supra* at 355-56, 636-37, 990-91, and 1154; for N-acylamides, *see*, March *supra* at 379; for N-Mannich bases, *see*, March *supra* at 800-02, and 828; for hydroxymethyl ketone esters, *see*, Petracek *et al. Annals NY Acad. Sci.*, **507**:353-54 (1987); for disulfides, *see*, March *supra* at 1160; and for phosphonate esters and phosphonamides.

The reactive functional groups can be chosen such that they do not participate in, or interfere with, the reactions necessary to assemble the reactive ligand analogue. Alternatively, a reactive functional group can be protected from participating in the reaction by the presence of a protecting group. Those of skill in the art will understand how to protect a particular functional group from interfering with a chosen set of reaction conditions. For examples of useful protecting groups, *see* Greene *et al.*, PROTECTIVE GROUPS IN ORGANIC SYNTHESIS, John Wiley & Sons, New York, 1991.

Generally, prior to forming the linkage between the ligand and the targeting (or other) agent, and optionally, the linker group, at least one of the chemical functionalities is activated. One skilled in the art will appreciate that a variety of chemical functionalities, including hydroxy, amino, and carboxy groups, can be activated using a variety of standard methods and conditions. For example, a hydroxyl group of the ligand (or targeting agent) can be activated through treatment with phosgene to form the corresponding chloroformate, or p-nitrophenylchloroformate to form the corresponding carbonate.

In an exemplary embodiment, the invention makes use of a targeting agent that includes a carboxyl functionality. Carboxyl groups may be activated by, for example, conversion to the corresponding acyl halide or active ester. This reaction may be performed under a variety of conditions as illustrated in March, *supra* pp. 388-89. In an exemplary embodiment, the acyl halide is prepared through the reaction of the carboxyl-containing group with oxalyl chloride. The activated agent is combined with a ligand or ligand-linker arm combination to form a conjugate of the invention. Those of skill in the art will appreciate that the use of carboxyl-containing targeting agents is merely illustrative, and that agents having many other functional groups can be conjugated to the ligands of the invention.

Targeting Groups

The compounds of the invention may also be conjugated to an agent that targets the compound to a specific tissue or region of disease. The compound of the invention can be targeted for specific delivery to the cells to be treated by conjugation of the compounds to a targeting agent. The term "targeting agent" refers to a species that serves to deliver the compound of the invention to a specific site. Targeting agents include, for example, molecules that specifically bind molecules present on a cell surface. Such targeting agents useful in the invention include anti-cell surface antigen antibodies; cytokines, including interleukins, factors such as epidermal growth factor (EGF), and the like, are also specific targeting agents known to bind cells expressing high levels of their receptors. Targeting agents include species that are taken up by cells using either active or passive mechanisms.

Particularly useful targeting agents for targeting the compounds of the invention to cells for therapeutic activity include those ligands that bind antigens or receptors present on virus-infected cells to be treated. For example, antigens present on T-cells, such as CD48, can be targeted with antibodies. Antibody fragments, including single chain fragments, can also be used. Other such ligand-receptor binding pairs are known in the scientific literature for targeting anti-viral treatments to target cells. Methods for producing conjugates of the compounds of the invention and the targeting moieties are known.

Membrane translocation polypeptides are another exemplary targeting agent. Membrane translocation polypeptides" have amphiphilic or hydrophobic amino acid subsequences that have the ability to act as membrane-translocating carriers. In one embodiment, homeodomain proteins have the ability to translocate across cell membranes. The shortest internalizable peptide of a homeodomain protein, Antennapedia, was found to be the third helix of the protein, from amino acid position 43 to 58 (*see, e.g., Prochiantz, Current Opinion in Neurobiology* 6:629-634 (1996)). Another subsequence, the h (hydrophobic) domain of signal peptides, was found to have similar cell membrane translocation characteristics (*see, e.g., Lin et al., J. Biol. Chem.* 270:1 4255-14258 (1995)).

Examples of peptide sequences include, but are not limited to: an 11 amino acid peptide of the tat protein of HIV; a 20 residue peptide sequence which corresponds to amino acids 84-103 of the p16 protein (*see Fahraeus et al., Current Biology* 6:84 (1996)); the third helix of the 60-amino acid long homeodomain of Antennapedia (Derossi *et al., J. Biol. Chem.* 269:10444 (1994)); the h region of a signal peptide such as the Kaposi fibroblast growth factor (K-FGF) h region (Lin *et al., supra*); or the VP22 translocation domain from

HSV (Elliot & O'Hare, *Cell* 88:223-233 (1997)). Other suitable chemical moieties that provide enhanced cellular uptake may also be chemically linked to the compounds of the invention.

Such subsequences can be used to translocate compounds of the invention across a cell membrane. Compounds of the invention can be conveniently fused to or derivatized with such sequences. Typically, the translocation sequence is provided as part of a fusion protein. Optionally, a linker as described herein can be used to link the compound of the invention and the translocation sequence. Any suitable linker can be used, *e.g.*, a peptide linker or other chemical linkers.

Toxin molecules also have the ability to transport compounds across cell membranes. Often, such molecules are composed of at least two parts (called "binary toxins"): a translocation or binding domain or polypeptide and a separate toxin domain or polypeptide. Typically, the translocation domain or polypeptide binds to a cellular receptor, and then the toxin is transported into the cell. Several bacterial toxins, including *Clostridium perfringens* iota toxin, diphtheria toxin (DT), *Pseudomonas* exotoxin A (PE), pertussis toxin (PT), *Bacillus anthracis* toxin, and pertussis adenylate cyclase (CYA), have been used in attempts to deliver peptides to the cell cytosol as internal or amino-terminal fusions (Arora et al., *J. Biol. Chem.*, 268: 3334-3341 (1993); Perelle et al., *Infect. Immun.*, 61: 5147-5156 (1993); Stenmark et al., *J. Cell Biol.* 113: 1025-1032 (1991); Donnelly et al., *PNAS U.S.A.* 90: 3530-3534 (1993); Carbonetti et al., *Abstr. Annu. Meet. Am. Soc. Microbiol.* 95: 295 (1995); Sebo et al., *Infect. Immun.* 63:3851-3857 (1995); Klimpel et al., *PNAS U.S.A.* 89:10277-10281 (1992); and Novak et al., *J. Biol. Chem.* 267:17186-17193 (1992)).

Non-covalent protein binding groups are also of use to target the compounds of the invention to specific regions of the body and to increase the half-life of the agent through protein binding.

Macromolecular Conjugates

In an exemplary embodiment, the invention provides a macromolecular, *i.e.*, MW > 1000 D, conjugate between the quinolone core and a macromolecular species. In one embodiment, a macromolecular conjugate of the invention is formed by covalently conjugating a quinolone to a macromolecule via a reactive functional group. In another embodiment, the macromolecular conjugate is formed by a non-covalent interaction between a quinolone derivative and a macromolecule, *e.g.*, a serum protein.

In the following discussion, the invention is described by reference to specific macromolecules of use for forming conjugates with the novel quinolone cores of the invention. Those of skill in the art will appreciate that the focus of the discussion is for clarity of illustration and does not limit the scope of the invention. The invention provides
5 macromolecular conjugates that include components derived from biomolecules and synthetic molecules. Exemplary biomolecules include polypeptides (e.g., antibodies, enzymes, receptors, antigens); polysaccharides (e.g., starches, inulin, dextran); lectins, non-peptide antigens and the like. Exemplary synthetic polymers include poly(acrylic acid), poly(lysine), poly(glutamic acid), poly(ethylene imine), etc.

Covalent Conjugation

Selection of an appropriate reactive functional group on a quinolone core of the invention to form a desired macromolecular species is well within the abilities of one of skill in the art. Exemplary reactive functional groups of use in forming the covalent
15 conjugates of the invention are discussed above. It is well within the abilities of one of skill to select and prepare a quinolone core of the invention having an appropriate reactive functional group of complementary reactivity to a reactive group on its conjugation partner.

In one embodiment, the bond formed between reactive functional groups of the macromolecule and that of the quinolone attaches the quinolone to the macromolecule
20 essentially irreversibly via a "stable bond" between the components. A "stable bond", as used herein, is a bond, which maintains its chemical integrity over a wide range of conditions (*e.g.*, amide, carbamate, carbon-carbon, ether, *etc.*). In another embodiment, a "cleaveable bond" links the macromolecule and the quinolone. A "cleaveable bond", as used herein, is a bond that undergoes scission under selected conditions. Cleaveable bonds include, but are not
25 limited to, disulfide, imine, carbonate and ester bonds. As discussed in the preceding sections, the reactive functional group can be located at one or more positions of the quinolone.

Polysaccharides

In an exemplary embodiment, the present invention provides conjugates
30 between a quinolone core and saccharides, *e.g.*, polysaccharides. In an exemplary embodiment, the invention provides a conjugate between a quinolone and inulin. Inulin is a naturally occurring polysaccharide, which has been previously investigated as a carrier for diagnostic moieties (Rongved, P. K., *J. Carbohydr. Res.* **1991**, *214*, 315; Corsi, D. M. V. E. et

al., *Chem. Eur. J.* **2001**, 7, 64). The structure of inulin can be described as a mixture of linear β -(2 \rightarrow 1)-linked α -D-fructofuranosyl chains with a α -D-glucopyranosyl unit at the terminal end. Inulin is commercially available in a variety of molecular weights and the degree of polymerization varies from 10 to 30, resulting in a molecular weight distribution of 1500 to 5000 Da. The high hydrophilicity, pH stability, low solution viscosity and biocompatibility of inulin ensure that its conjugates have favorable pharmacological properties.

Dendrimer-Based Agents

In another aspect, the present invention provides a quinolone as set forth above, which is attached to a dendrimer via a reactive functional group. Similar to the polymeric group discussed above, the dendrimer has at least two reactive functional groups. In one embodiment, one or more formed quinolone is attached to the dendrimer. Alternatively, the quinolone is formed directly on the dendrimer.

In an exemplary embodiment, a water-soluble and bio-adapted polyester (polypropionate) class of dendrimers has been designed to provide favorable pharmacokinetic properties. See, for example, Ihre, H. et al., *Macromolecules* **1998**, 31, 4061; Ihre, H. et al., *J. Am. Chem. Soc.* **1996**, 118, 6388; Anders, H., Ihre, H., Patent W0/9900440 (Sweden)). In an exemplary embodiment, the termini of the dendrimers are conjugated to a quinolone core of the invention.

Poly(ethylene glycol)-based Agents

In another exemplary embodiment, the invention provides a conjugate between a quinolone core of the invention and poly(ethylene glycol). Poly(ethylene glycol) (PEG) is used in biotechnology and biomedical applications. The use of this agent has been reviewed (POLY(ETHYLENE GLYCOL) CHEMISTRY: BIOTECHNICAL AND BIOMEDICAL APPLICATIONS, J. M. Harris, Ed., Plenum Press, New York, 1992). Modification of enzymes (Chiu et al., *J. Bioconjugate Chem.*, 4: 290-295 (1993)), RGD peptides (Braatz et al., *Bioconjugate Chem.*, 4: 262-267 (1993)), liposomes (Zalipsky, *S. Bioconjugate Chem.*, 4: 296-299 (1993)), and CD4-IgG glycoprotein (Chamow et al., *Bioconjugate Chem.*, 4: 133-140 (1993)) are some of the recent advances in the use of polyethylene glycol. Surfaces treated with PEG have been shown to resist protein deposition and have improved resistance to thrombogenicity when coated on blood contacting biomaterials (Merrill, "Poly(ethylene oxide) and Blood Contact: A Chronicle of One Laboratory," in POLY(ETHYLENE GLYCOL)

CHEMISTRY: BIOTECHNICAL AND BIOMEDICAL APPLICATIONS, Harris, Ed., Plenum Press, New York, (1992), pp. 199-220).

Many routes are available for attaching a quinolone core of the invention onto a polymeric or oligomeric species. See, for example, Dunn, R.L., *et al.*, Eds. POLYMERIC
5 DRUGS AND DRUG DELIVERY SYSTEMS, ACS Symposium Series Vol. 469, American Chemical Society, Washington, D.C. 1991; Herren *et al.*, *J. Colloid and Interfacial Science* **115**: 46-55 (1987); Nashabeh *et al.*, *J. Chromatography* **559**: 367-383 (1991); Balachandar *et al.*, *Langmuir* **6**: 1621-1627 (1990); and Burns *et al.*, *Biomaterials* **19**: 423-440 (1998).

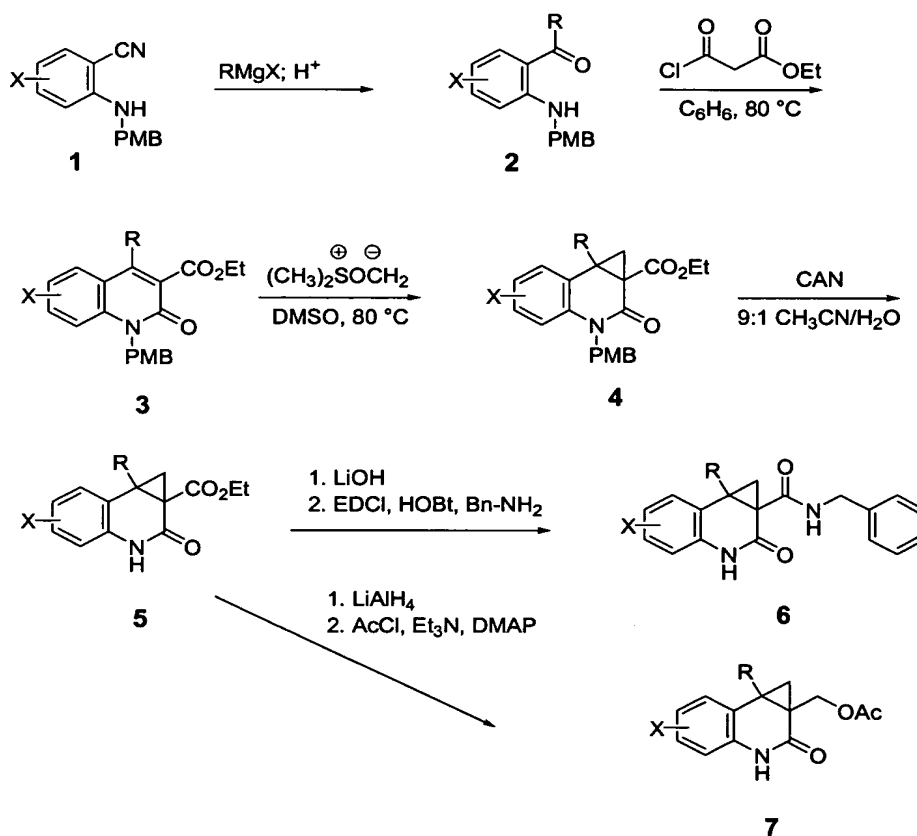
Many activated derivatives of poly(ethyleneglycol) are available commercially
10 and in the literature. It is well within the abilities of one of skill to choose, and synthesize if necessary, an appropriate activated PEG derivative with which to prepare a conjugate useful in the present invention. See, Abuchowski *et al.* *Cancer Biochem. Biophys.*, **7**: 175-186 (1984); Abuchowski *et al.*, *J. Biol. Chem.*, **252**: 3582-3586 (1977); Jackson *et al.*, *Anal. Biochem.*, **165**: 114-127 (1987); Koide *et al.*, *Biochem Biophys. Res. Commun.*, **111**: 659-667
15 (1983)), tresylate (Nilsson *et al.*, *Methods Enzymol.*, **104**: 56-69 (1984); Delgado *et al.*, *Biotechnol. Appl. Biochem.*, **12**: 119-128 (1990)); N-hydroxysuccinimide derived active esters (Buckmann *et al.*, *Makromol. Chem.*, **182**: 1379-1384 (1981); Joppich *et al.*, *Makromol. Chem.*, **180**: 1381-1384 (1979); Abuchowski *et al.*, *Cancer Biochem. Biophys.*, **7**: 175-186 (1984); Katre *et al.* *Proc. Natl. Acad. Sci. U.S.A.*, **84**: 1487-1491 (1987); Kitamura *et al.*, *Cancer Res.*, **51**: 4310-4315 (1991); Boccu *et al.*, *Z. Naturforsch.*, **38C**: 94-99 (1983),
20 carbonates (Zalipsky *et al.*, POLY(ETHYLENE GLYCOL) CHEMISTRY: BIOTECHNICAL AND BIOMEDICAL APPLICATIONS, Harris, Ed., Plenum Press, New York, 1992, pp. 347-370; Zalipsky *et al.*, *Biotechnol. Appl. Biochem.*, **15**: 100-114 (1992); Veronese *et al.*, *Appl. Biochem. Biotech.*, **11**: 141-152 (1985)), imidazolyl formates (Beauchamp *et al.*, *Anal. Biochem.*, **131**: 25-33 (1983); Berger *et al.*, *Blood*, **71**: 1641-1647 (1988)), 4-dithiopyridines (Woghiren *et al.*, *Bioconjugate Chem.*, **4**: 314-318 (1993)), isocyanates (Byun *et al.*, *ASAIO Journal*, M649-M-653 (1992)) and epoxides (U.S. Pat. No. 4,806,595, issued to Noishiki *et al.*, (1989). Other linking groups include the urethane linkage between amino groups and activated PEG. See, Veronese, *et al.*, *Appl. Biochem. Biotechnol.*, **11**: 141-152 (1985).

30 **Synthesis and Purification of Quinolones**

The compounds of the invention are synthesized by an appropriate combination of generally well-known synthetic methods. Techniques useful in synthesizing the compounds of the invention are both readily apparent and accessible to those of skill in

the relevant art. The discussion below is offered to illustrate certain of the diverse methods available for use in assembling the compounds of the invention, it is not intended to define the scope of reactions or reaction sequences that are useful in preparing the compounds of the present invention. Exemplary reaction schemes leading to the formation of quinolones of the invention are set forth below.

Scheme 1



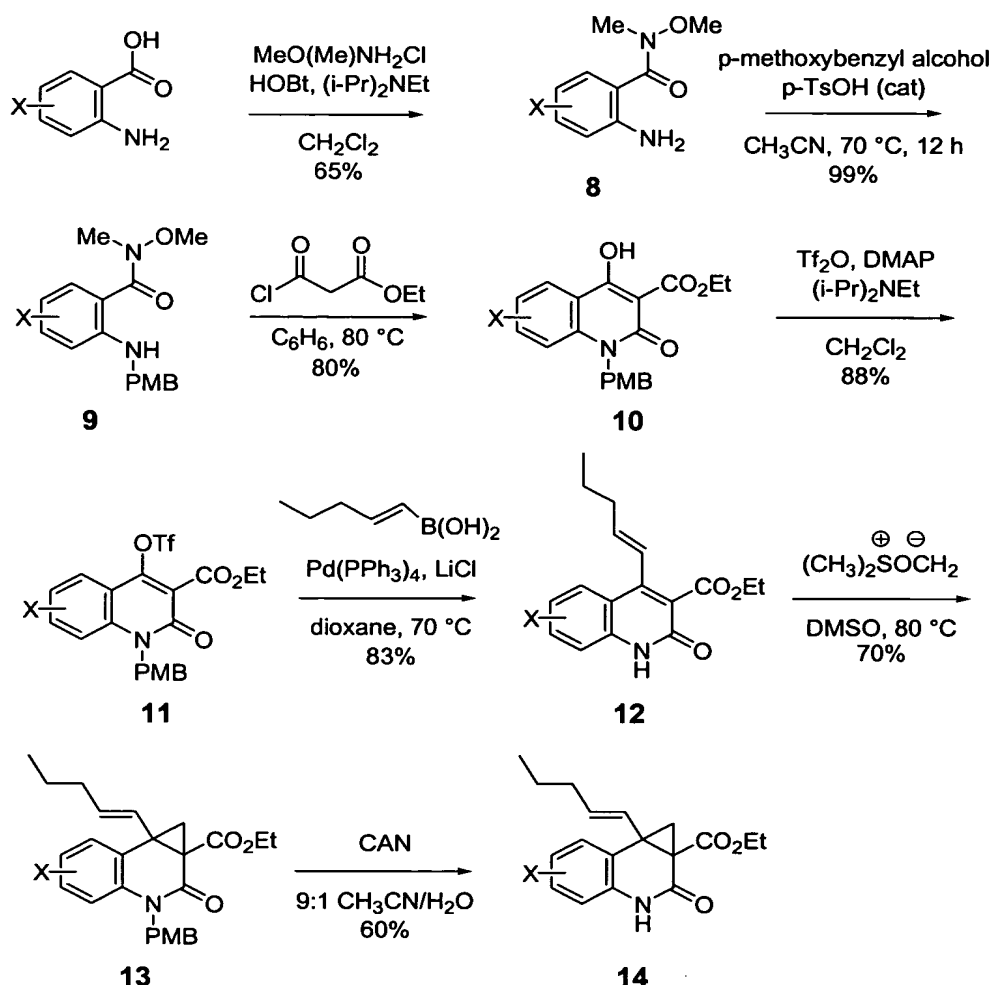
One method of synthesizing the compounds of the invention is set forth in Scheme 1. In an exemplary process according to Scheme 1, the *p*-methoxybenzyl (hereinafter referred to as "PMB") aniline 1 is prepared from commercially available 2-amino-4-chlorobenzonitrile. Treatment of 1 with a Grignard reagent followed by acid catalyzed hydrolysis affords the desired PMB ketoaniline 2. Reaction of 2 with ethyl-3-chloro-3-oxopropionate provides the α,β-unsaturated-β-amidoester 3. Cyclopropanation of 3 with dimethyloxosulfonium methylide gives PMB quinolone 4 in 50–85% yield. Compound 4 is then deprotected with ammonium cerium (IV) nitrate (CAN) to give the desired quinolone 5.

The ester substituent in 5 is hydrolyzed to give the corresponding carboxylic acid, followed by addition of benzyl amine to yield the corresponding quinolone amide 6.

Alternatively, the ester substituent in **5** is reduced to give the corresponding alcohol, followed by addition of acetylchloride to form the corresponding ether **7**.

Scheme 2 sets forth an exemplary route for preparing compounds in which a carbon atom joining the cyclopropyl moiety to the heterocyclic ring system is substituted with a variety of substituted or unsubstituted alkyl moieties.

Scheme 2

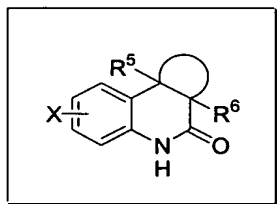


Thus, the aminobenzoic acid starting material is converted to the corresponding N-alkoxycarboxamide **8** by amidation of the carboxylic acid. The amine moiety of compound **8** is protected, providing **9**. Cyclization of the protected amine derivative provides the corresponding fused heterocyclic ring system **10**. The hydroxyl moiety of **10** is converted to the OTf moiety by the action of Tf_2O , and is subsequently displaced by an alkyl boronic acid, affording **12**.

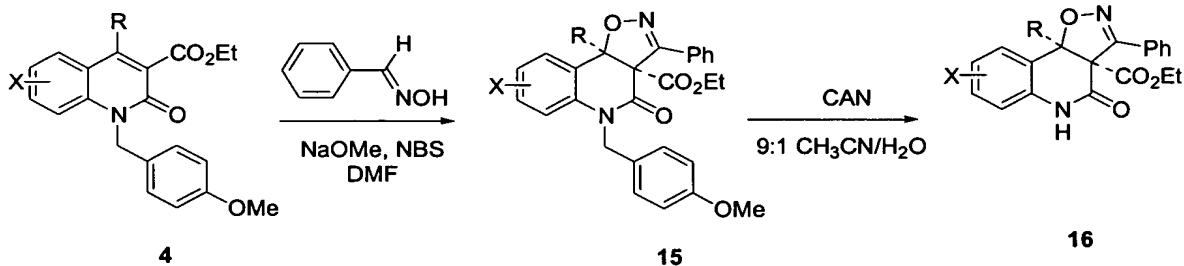
Scheme 3 sets forth an exemplary synthetic route to compounds of the invention in which the cyclopropyl ring is replaced with a different ring system. Scheme 3a

provides a route to fused ring systems of the invention having two heteroatoms, such as oxazoles, thiazoles, pyrazoles and the like. Scheme 3b illustrates a route for preparing compounds of the invention in which the fused ring system incorporates a cycloalkyl ring that having more than three carbon atoms.

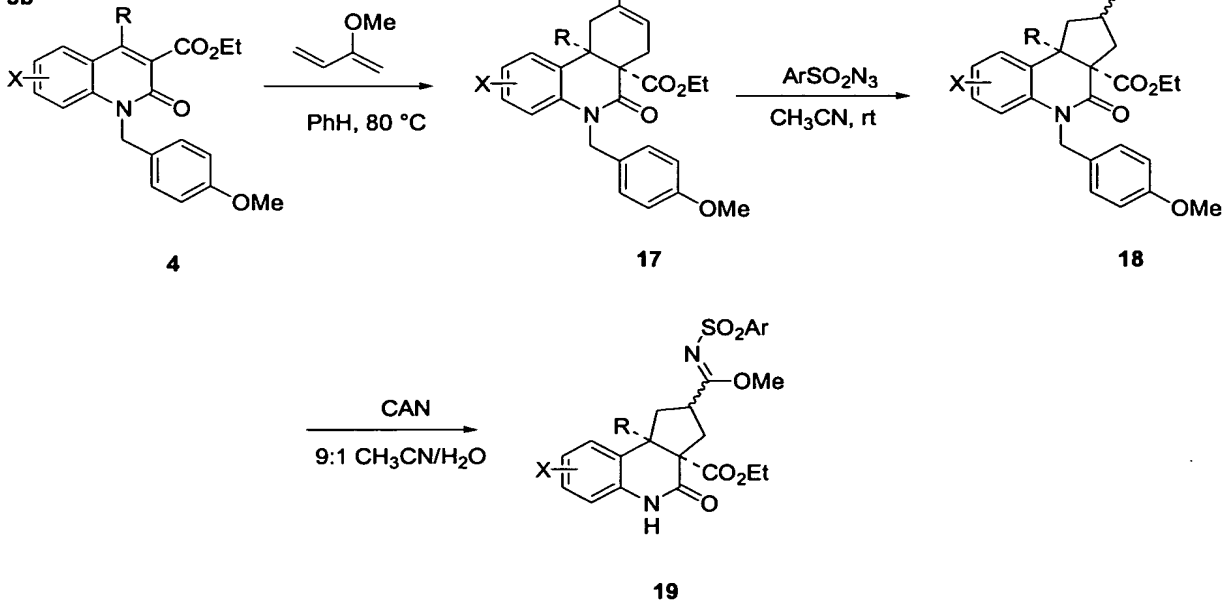
5 Scheme 3



3a



3b

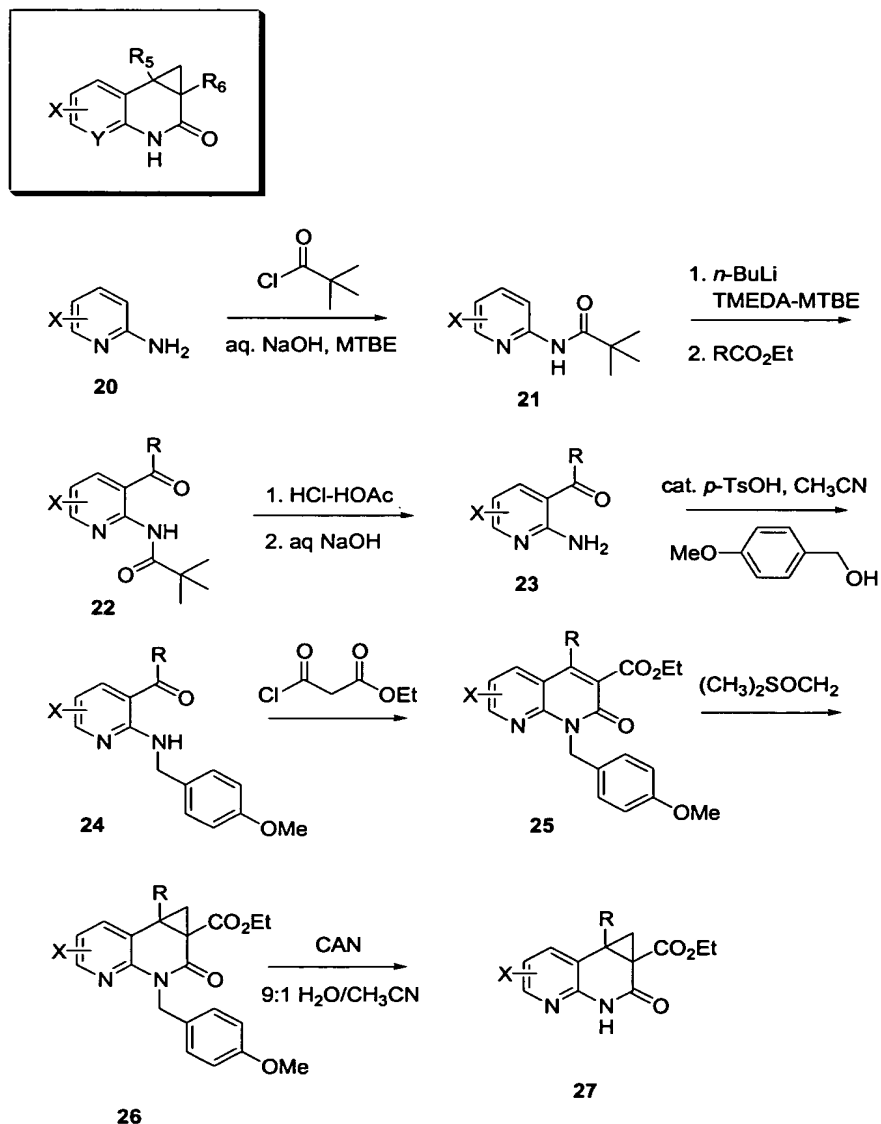


In Scheme 3a, unsaturated ester **4** is cyclized to the corresponding oxazole **15**, which is deprotected to afford quinolone **16**.

In Scheme 3b, starting ester **4** is cyclized to a ring system with two fused six-member rings, **17**. Compound **17** undergoes a ring contraction reaction to provide compound **18**, having fused 5- and 6-membered rings, which is deprotected to provide quinolone **19**.

Scheme 4 provides an exemplary synthetic scheme for compounds of the invention in which the phenyl ring is replaced by a heterocyclic system, exemplified by a pyridyl system.

Scheme 4



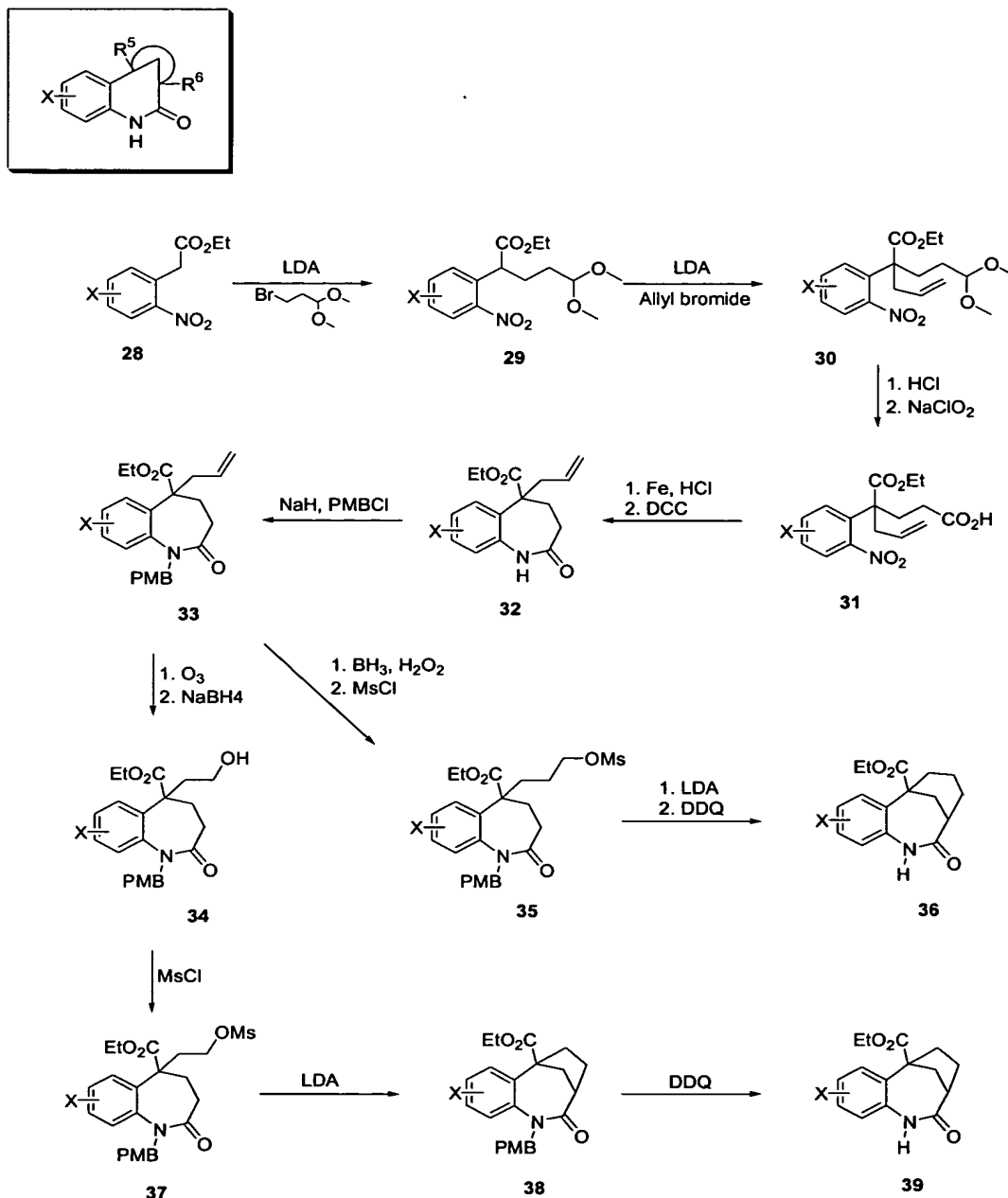
In Scheme 4, the amine moiety of starting aminopyridine **20** is acylated, affording compound **21**, which is subsequently carbonylated to compound **22**. The amide moiety of **22** is converted to the corresponding amine by the action of HCl/HOAc, affording **23**. The amine moiety of **23** is protected with the PMB group in compound **24** and the fused

ring system of compound **25** is prepared. Contacting **24** with an ylide reagent provides the cyclopropyl structure of compound **26**, which is deprotected to afford compound **27**.

Scheme 5 sets forth an exemplary route that provides oxindoles of the invention in which two of the ring systems are varied in size.

5

Scheme 5



In Scheme 5, an aldol protected 3-carbon fragment is added to starting material **28**, affording **29**. Allyl bromide provides an allyl moiety, forming compound **30**. The aldol moiety is removed and the resulting aldehyde is oxidized to the corresponding carboxylic acid **31**. The nitro moiety is reduced to the amine, which is coupled to the

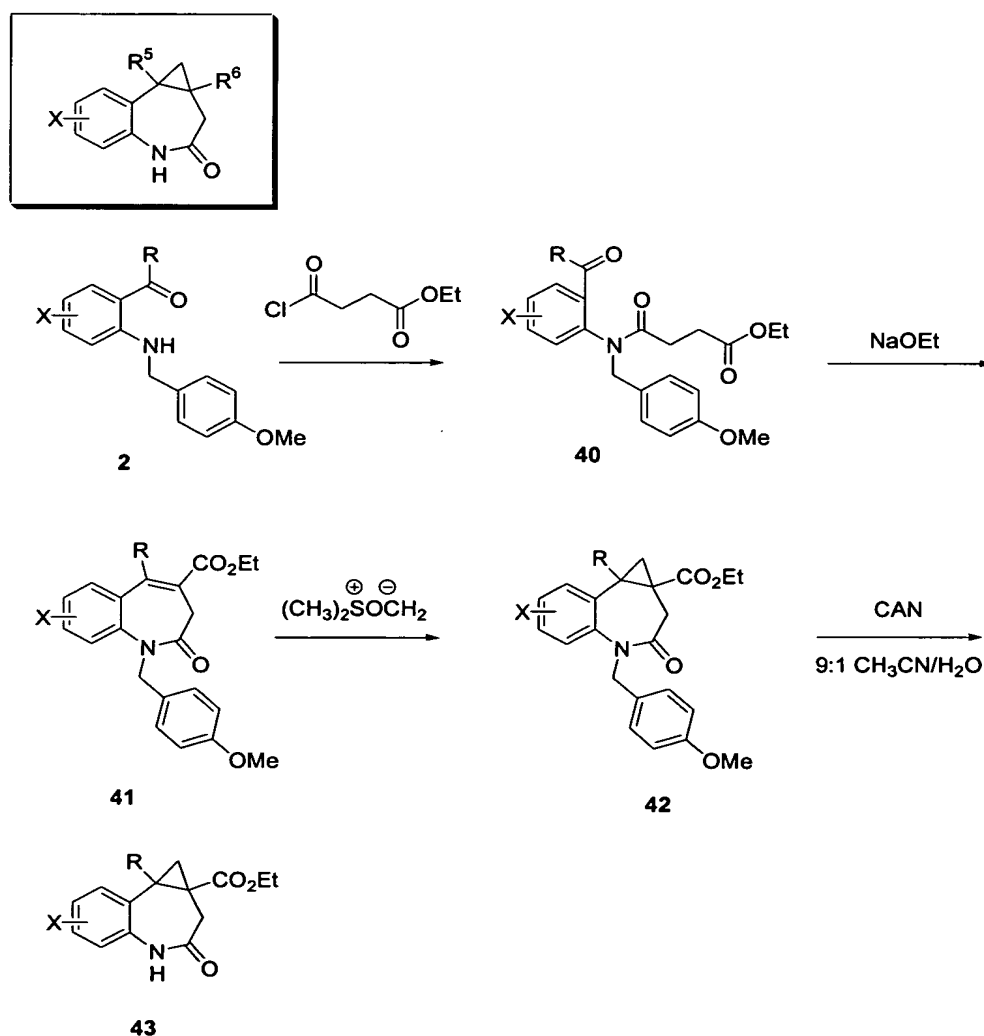
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carboxylic acid by the action of DCC, forming **32**. The nitrogen of the resulting lactam is protected as the PMB derivative in **33**, which is ozonized to convert the double bond of the allyl-derived double bond to a hydroxyl, which in **34**, is attached to the ring system through a two-carbon linker. The hydroxyl group is converted to a mesylate **37**, which is cyclized to compound **38** by the action of LDA. Compound **38** is deprotected using DDQ.

Alternatively, the double bond is converted to a hydroxyl linked to the ring system by a three-carbon linker via the action of BH_3 and H_2O_2 , followed by its conversion to the corresponding mesyl group of **35**, which is cyclized and deprotected to provide **36**.

Scheme 6 provides an exemplary route to compounds of the invention in which the cyclic lactam ring system is varied in size.

Scheme 6



In Scheme 6, PMB-protected starting material **2** is converted to amide-ester **40**, which is cyclized to the seven-member lactam ring of **41**. The cyclopropyl ring of **42** is

formed by contacting **41** with an ylide reagent. Removing the PMB protecting group affords **43**.

Diversification of the final quinolone product is obtained by making modifications to the above exemplary schemes. For example, the Grignard reagent may comprise a wide variety of R substituents. Such substituents include, but are not limited to substituted or unsubstituted alkyls, aryls, heteroaryls, or heterocycloalkyls. In addition, the ester provides an ideal handle for increasing diversity, e.g., final diversity. Exemplary compounds produced by such modifications are presented in **FIG. 1**.

The compounds of the invention are synthesized by either solution phase or solid phase synthesis. The solid support used is not a critical feature of the present invention provided that it is capable of binding to the carboxyl group while remaining substantially inert to the reagents utilized in the peptide synthesis procedure. For example, a starting material can be prepared by attaching an amino-protected precursor via a benzyl ester linkage to a chloromethylated resin or a hydroxymethyl resin or via an amide bond to a benzhydrylamine (BHA) resin or p-methylbenzhydrylamine (MBHA) resin. Materials suitable for use as solid supports are well known to those of skill in the art and include, but are not limited to, the following: halomethyl resins, such as chloromethyl resin or bromomethyl resin; hydroxymethyl resins; phenol resins, such as 4-(α -[2,4-dimethoxyphenyl]-Fmoc-aminomethyl)phenoxy resin; tert-alkyloxycarbonyl-hydrazidated resins, and the like. Such resins are commercially available and their methods of preparation are known to those of ordinary skill in the art.

The compounds of the present invention can be isolated and purified from the reaction mixture by means of purification strategies well known to those of skill in the art. For example, the compounds may be purified using known chromatographic procedures such as reverse phase HPLC, gel permeation, ion exchange, size exclusion, affinity, partition, or countercurrent distribution.

Pharmaceutical Formulations

The compounds of the present invention can be prepared and administered in a wide variety of oral, parenteral and topical dosage forms. Thus, the compounds of the present invention can be administered by injection, that is, intravenously, intramuscularly, intracutaneously, subcutaneously, intraduodenally, or intraperitoneally. Also, the compounds described herein can be administered by inhalation, for example, intranasally. Additionally, the compounds of the present invention can be administered transdermally. Accordingly, the

present invention also provides pharmaceutical compositions comprising a pharmaceutically acceptable carrier or excipient and one or more compounds of the invention.

For preparing pharmaceutical compositions from the compounds of the present invention, pharmaceutically acceptable carriers can be either solid or liquid. Solid form preparations include powders, tablets, pills, capsules, cachets, suppositories, and dispersible granules. A solid carrier can be one or more substances, which may also act as diluents, flavoring agents, binders, preservatives, tablet disintegrating agents, or an encapsulating material.

In powders, the carrier is a finely divided solid, which is in a mixture with the finely divided active component. In tablets, the active component is mixed with the carrier having the necessary binding properties in suitable proportions and compacted in the shape and size desired.

The powders and tablets preferably contain from 5% or 10% to 70% of the active compound. Suitable carriers are magnesium carbonate, magnesium stearate, talc, sugar, lactose, pectin, dextrin, starch, gelatin, tragacanth, methylcellulose, sodium carboxymethylcellulose, a low melting wax, cocoa butter, and the like. The term "preparation" is intended to include the formulation of the active compound with encapsulating material as a carrier providing a capsule in which the active component with or without other carriers, is surrounded by a carrier, which is thus in association with it. Similarly, cachets and lozenges are included. Tablets, powders, capsules, pills, cachets, and lozenges can be used as solid dosage forms suitable for oral administration.

For preparing suppositories, a low melting wax, such as a mixture of fatty acid glycerides or cocoa butter, is first melted and the active component is dispersed homogeneously therein, as by stirring. The molten homogeneous mixture is then poured into convenient sized molds, allowed to cool, and thereby to solidify.

Liquid form preparations include solutions, suspensions, and emulsions, for example, water or water/propylene glycol solutions. For parenteral injection, liquid preparations can be formulated in solution in aqueous polyethylene glycol solution.

Aqueous solutions suitable for oral use can be prepared by dissolving the active component in water and adding suitable colorants, flavors, stabilizers and thickening agents as desired. Aqueous suspensions suitable for oral use can be made by dispersing the finely divided active component in water with viscous material, such as natural or synthetic gums, resins, methylcellulose, sodium carboxymethylcellulose and other well-known suspending agents.

Also included are solid form preparations, which are intended to be converted, shortly before use, to liquid form preparations for oral administration. Such liquid forms include solutions, suspensions, and emulsions. These preparations may contain, in addition to the active component, colorants, flavors, stabilizers, buffers, artificial and natural
5 sweeteners, dispersants, thickeners, solubilizing agents, and the like.

The dose administered to a patient, in the context of the present invention should be sufficient to provide a beneficial therapeutic response in the patient over time. The dose is determined by the efficacy of the particular compound employed and the condition of the patient, as well as the body weight or surface area of the patient to be treated. The size of
10 the dose also is determined by the existence, nature, and extent of any adverse side effects that accompany the administration of a particular compound in a particular patient.

The compound can also be introduced into an animal cell, preferably a mammalian cell, via a microparticles and liposomes and liposome derivatives such as immunoliposomes. The term "liposome" refers to vesicles comprised of one or more
15 concentrically ordered lipid bilayers, which encapsulate an aqueous phase. The aqueous phase typically contains the compound to be delivered to the cell.

The liposome fuses with the plasma membrane, thereby releasing the drug into the cytosol. Alternatively, the liposome is phagocytosed or taken up by the cell in a transport vesicle. Once in the endosome or phagosome, the liposome either degrades or fuses with the
20 membrane of the transport vesicle and releases its contents.

In current methods of drug delivery via liposomes, the liposome ultimately becomes permeable and releases the encapsulated compound at the target tissue or cell. For systemic or tissue specific delivery, this can be accomplished, for example, in a passive manner wherein the liposome bilayer degrades over time through the action of various agents
25 in the body. Alternatively, active drug release involves using an agent to induce a permeability change in the liposome vesicle. Liposome membranes can be constructed so that they become destabilized when the environment becomes acidic near the liposome membrane (*see, e.g., PNAS* 84:7851 (1987); *Biochemistry* 28:908 (1989)). When liposomes are endocytosed by a target cell, for example, they become destabilized and release their
30 contents. This destabilization is termed fusogenesis. Dioleoylphosphatidyl-ethanolamine (DOPE) is the basis of many "fusogenic" systems.

Such liposomes typically comprise a compound of choice and a lipid component, *e.g.*, a neutral and/or cationic lipid, optionally including a receptor-recognition molecule such as an antibody that binds to a predetermined cell surface receptor or ligand

(e.g., an antigen). A variety of methods are available for preparing liposomes as described in, e.g., Szoka *et al.*, *Ann. Rev. Biophys. Bioeng.* 9:467 (1980), U.S. Pat. Nos. 4,186,183, 4,217,344, 4,235,871, 4,261,975, 4,485,054, 4,501,728, 4,774,085, 4,837,028, 4,235,871, 4,261,975, 4,485,054, 4,501,728, 4,774,085, 4,837,028, 4,946,787, PCT Publication No. WO 91\17424, Deamer & Bangham, *Biochim. Biophys. Acta* 443:629-634 (1976); Fraley, *et al.*, *PNAS* 76:3348-3352 (1979); Hope *et al.*, *Biochim. Biophys. Acta* 812:55-65 (1985); Mayer *et al.*, *Biochim. Biophys. Acta* 858:161-168 (1986); Williams *et al.*, *PNAS* 85:242-246 (1988); *Liposomes* (Ostro (ed.), 1983, Chapter 1); Hope *et al.*, *Chem. Phys. Lip.* 40:89 (1986); Gregoriadis, *Liposome Technology* (1984) and Lasic, *Liposomes: from Physics to Applications* (1993)). Suitable methods include, for example, sonication, extrusion, high pressure/homogenization, microfluidization, detergent dialysis, calcium-induced fusion of small liposome vesicles and ether-fusion methods, all of which are well known in the art.

In certain embodiments of the present invention, it is desirable to target the liposomes of the invention using targeting moieties that are specific to a particular cell type, tissue, and the like. Targeting of liposomes using a variety of targeting moieties (e.g., ligands, receptors, and monoclonal antibodies) has been previously described (*see, e.g.*, U.S. Patent Nos. 4,957,773 and 4,603,044).

Standard methods for coupling targeting agents to liposomes can be used. These methods generally involve incorporation into liposomes lipid components, e.g., phosphatidylethanolamine, which can be activated for attachment of targeting agents, or derivatized lipophilic compounds, such as lipid-derivatized bleomycin. Antibody targeted liposomes can be constructed using, for instance, liposomes which incorporate protein A (*see Renneisen et al.*, *J. Biol. Chem.*, 265:16337-16342 (1990) and Leonetti *et al.*, *PNAS* 87:2448-2451 (1990)).

The pharmaceutical preparation is preferably in unit dosage form. In such form the preparation is subdivided into unit doses containing appropriate quantities of the active component. The unit dosage form can be a packaged preparation, the package containing discrete quantities of preparation, such as packeted tablets, capsules, and powders in vials or ampoules. Also, the unit dosage form can be a capsule, tablet, cachet, or lozenge itself, or it can be the appropriate number of any of these in packaged form. The quantity of active component in a unit dose preparation may be varied or adjusted from 0.1 mg to 10 g, more typically 1.0 mg to 1 g, most typically 10 mg to 500 mg, according to the particular application and the potency of the active component. The composition can, if desired, also

contain other compatible therapeutic or diagnostic agents. Administration can be accomplished via single or divided doses.

The Methods

5 The present invention also provides methods for treating or ameliorating HIV disease and related diseases. The method includes administering a therapeutically effective dosage of at least one compound of the invention to a subject suffering from HIV disease or HIV-related diseases. The invention also provides a method of combination therapy in which one or more compound of the invention is administered in combination with one or more
10 other compound having activity against HIV disease or HIV-related disease.

Dosage levels of approximately 0.02 to approximately 10.0 grams of a compound of the invention per day are useful in the treatment or prevention of retroviral infection, such as HIV infection, AIDS or AIDS-related complex (ARC), with oral doses 2 to 5 times higher. For example, HIV infection can be treated by administration of from about
15 0.1 to about 100 milligrams of compound per kilogram of body weight from one to four times per day. In one embodiment, dosages of about 100 to about 400 milligrams of compound are administered orally every six hours to a subject. The specific dosage level and frequency for any particular subject is varied and will depend upon a variety of factors, including the activity of the specific compound the metabolic stability and length of action of that
20 compound, the age, body weight, general health, sex, and diet of the subject, mode of administration, rate of excretion, drug combination, and severity of the particular condition.

The invention provides methods for inhibiting the replication of drug resistant HIV mutants. The high replication rate of HIV leads to genetic variants (mutants), especially when selective pressure is introduced in the form of drug treatment. The mutants frequently
25 display resistance to anti-viral agents previously administered to the patient. Switching agents or using combination therapies may decrease or delay resistance, but because viral replication is not completely suppressed in single drug treatment or even with a two-drug combination, drug-resistant viral strains ultimately emerge. Triple drug combinations employing one (or two) nucleoside analogs and two (or one) non-nucleoside inhibitor (NNI)
30 targeting RT provide a very promising therapy to overcome the drug resistance problem. RT mutant strains resistant to such a triple action drug combination would most likely not be able to function.

Dozens of mutant strains have been characterized as resistant to NNI compounds, including L1001, K103N, V106A, E138K, Y181C and Y188H. In particular,

the Y181C and K103N mutants may be the most difficult to treat, because they are resistant to most of the NNI compounds that have been examined.

Combination Therapies

5 In numerous embodiments, the compounds of the present invention are administered in combination with one or more additional compounds or therapies. For example, multiple reverse transcriptase inhibitors can be co-administered, or one or more compound of the invention can be administered in conjunction with another therapeutic compound. In one embodiment, the other therapeutic agent is one that is used to prevent or
10 treat HIV infection. In another embodiment, the other therapeutic agent is an agent used to treat an opportunistic infection associated with HIV infection and/or to treat or prevent HIV infection.

 Suitable therapeutic agents for use in combination with the compounds of the present invention include, but are not limited to, protease inhibitors, non-nucleoside reverse
15 transcriptase inhibitors, nucleoside reverse transcriptase inhibitors, antiretroviral nucleosides, entry inhibitors as well as other anti-viral agents effective to inhibit or treat HIV infection. Further examples of suitable therapeutic agents include, but are not limited to, zidovudine, didanosine, stavudine, interferon, lamivudine, adefovir, nevirapine, delaviridine, loviride, saquinavir, indinavir, and AZT. Other suitable therapeutic agents include, but are not limited
20 to, antibiotics or other anti-viral agents, *e.g.*, acyclovir.

 Other combination therapies known to those of skill in the art can be used in conjunction with the compositions and methods of the present invention.

 As explained above, it has now been discovered that quinolones of the invention have anti-viral activity. As such, the compounds of the invention can be used to
25 inhibit a wide variety of viruses and, thus, to treat a wide variety of viral infections in a human. Viruses that can be inhibited using the compounds of the invention include, but are not limited, to DNA viruses, RNA viruses as well as retroviruses. Examples of viruses that can be inhibited using the compounds include, but are not limited to, Herpes viruses, Hepatitis (A, B and C) viruses, influenza viruses, POX viruses, Rhino viruses and HTLV
30 (Human T-cell Leukemia) viruses (*e.g.*, HTLV 1 and 2). Based on their anti-viral activity, those of skill in the art are aware of other viruses that can be treated using compounds of the invention.

Assays for Modulators of Reverse Transcriptase

Modulation of a reverse transcriptase, and corresponding modulation of HIV and viral infection, preferably inhibition, can be assessed using a variety of *in vitro* and *in vivo* assays, including cell-based models. Such assays can be used to test for inhibitors and
5 activators of reverse transcriptase, and, consequently, inhibitors and activators of HIV infection and HIV-associated diseases. Such modulators of reverse transcriptase are useful for treating disorders related to HIV infection, as described herein. Modulators of reverse transcriptase are tested using either recombinant, chemically synthesized or naturally occurring reverse transcriptase.

10 Preferred modulators of the invention are those that act to decrease reverse transcriptase activity at the protein level. Preferred modulators also include those that decrease expression of reverse transcriptase at the nucleic acid level, *e.g.*, inhibitors of the reverse transcriptase promoter, compounds that increase chromosome accessibility of the reverse transcriptase gene, compounds that decrease reverse transcriptase RNA stability and
15 processing, and compounds that decrease reverse transcriptase RNA levels in the cytoplasm or nucleus.

Measurement of HIV infection modulation with a reverse transcriptase inhibitor, can be performed using a variety of assays, *in vitro*, *in vivo*, and *ex vivo*, as described herein. A suitable physical, chemical or phenotypic change that affects activity,
20 *e.g.*, enzymatic activity, cell proliferation (*e.g.*, CD4⁺ lymphocyte proliferation), HIV replication, expression of HIV proteins, or ligand or substrate binding can be used to assess the influence of a test compound on the polypeptide of this invention. When the functional effects are determined using intact cells or animals, one can also measure a variety of effects, such as, viral RNA levels or viral titers in serum, ligand binding, transcriptional changes to
25 both known and uncharacterized genetic markers (*e.g.*, northern blots), changes in cell metabolism, changes related to cellular proliferation, viral marker expression, DNA synthesis, marker and dye dilution assays (*e.g.*, GFP and cell tracker assays), etc.

In vitro assays

30 Assays to identify compounds with reverse transcriptase modulating activity can be performed *in vitro*. As described below, the assay can be either solid state or soluble. The protein may be bound to a solid support, either covalently or non-covalently. Often, the *in vitro* assays of the invention are substrate or ligand binding or affinity assays, either non-competitive or competitive. Other *in vitro* assays include measuring changes in spectroscopic

(e.g., fluorescence, absorbance, refractive index), hydrodynamic (e.g., shape), chromatographic, or solubility properties for the protein.

In one embodiment, a high throughput-binding assay is performed in which the reverse transcriptase or a fragment thereof is contacted with a potential modulator and incubated for a suitable amount of time. In one embodiment, the potential modulator is bound to a solid support, and the reverse transcriptase is added. In another embodiment, the reverse transcriptase is bound to a solid support. A wide variety of assays can be used to identify reverse transcriptase-modulator binding, including labeled protein-protein binding assays, electrophoretic mobility shifts, immunoassays, enzymatic assays such as kinase assays, and the like. In some cases, the binding of the candidate modulator is determined through the use of competitive binding assays, where interference with binding of a known ligand or substrate is measured in the presence of a potential modulator. The modulator or the known ligand or substrate is bound first, and then the competitor is added. After the reverse transcriptase is washed, interference with binding, either of the potential modulator or of the known ligand or substrate, is determined. Often, either the potential modulator or the known ligand or substrate is labeled.

Cell-based in vivo assays

In another embodiment, reverse transcriptase is expressed in a cell, and functional, e.g., physical and chemical or phenotypic, changes are assayed to identify modulators of reverse transcriptase and modulators of HIV replication and HIV infected cells. Cells expressing reverse transcriptase can also be used in binding assays and enzymatic assays. Any suitable functional effect can be measured, as described herein. For example, cellular morphology (e.g., cell volume, nuclear volume, cell perimeter, and nuclear perimeter), ligand binding, lymphocyte proliferation, apoptosis, viral marker expression, GFP positivity and dye dilution assays (e.g., cell tracker assays with dyes that bind to cell membranes), DNA synthesis assays (e.g., ³H-thymidine and fluorescent DNA-binding dyes such as BrdU or Hoechst dye with FACS analysis), are all suitable assays to identify potential modulators using a cell based system. Suitable cells for such cell based assays include both primary cells such as PBMCs, lymphocytes (e.g., CD4+), neutrophils, polymorphonuclear leukocytes, and other phagocytic cells and cell lines, e.g., Jurkat cells, BJAB cells, etc. The reverse transcriptase can be naturally occurring or recombinant.

Cellular reverse transcriptase RNA and polypeptide levels can be determined by measuring the level of protein or mRNA. The level of reverse transcriptase or proteins

related to reverse transcriptase are measured using immunoassays such as western blotting, ELISA and the like with an antibody that selectively binds to the reverse transcriptase polypeptide or a fragment thereof. For measurement of mRNA, amplification, *e.g.*, using PCR, LCR, or hybridization assays, *e.g.*, northern hybridization, RNase protection, dot blotting, are preferred. The level of protein or mRNA is detected using directly or indirectly labeled detection agents, *e.g.*, fluorescently or radioactively labeled nucleic acids, radioactively or enzymatically labeled antibodies, and the like, as described herein.

Alternatively, reverse transcriptase expression can be measured using a reporter gene system, *e.g.*, utilizing a fusion protein or a gene linked to a reverse transcriptase promoter. Such a system can be devised using an reverse transcriptase protein promoter operably linked to a reporter gene such as chloramphenicol acetyltransferase, firefly luciferase, bacterial luciferase, β -galactosidase and alkaline phosphatase. Furthermore, the protein of interest can be used as an indirect reporter via attachment to a second reporter such as red or green fluorescent protein (*see, e.g.*, Mistili & Spector, *Nature Biotechnology* 15:961-964 (1997)). The reporter construct is typically transfected into a cell. After treatment with a potential modulator, the amount of reporter gene transcription, translation, or activity is measured according to standard techniques known to those of skill in the art.

Animal models

Animal models of HIV infection also find use in screening for modulators of reverse transcriptase. Similarly, transgenic animal technology including gene knockout technology, for example as a result of homologous recombination with an appropriate gene targeting vector, or gene overexpression, will result in the absence or increased expression of the reverse transcriptase. The same technology can also be applied to make knock-out cells. When desired, tissue-specific expression or knockout of the reverse transcriptase protein may be necessary.

Knock-out cells and transgenic mice can be made by insertion of a marker gene or other heterologous gene into an endogenous reverse transcriptase gene site in the mouse genome via homologous recombination. Such mice can also be made by substituting an endogenous reverse transcriptase with a mutated version of the reverse transcriptase gene by mutating an endogenous reverse transcriptase, *e.g.*, by exposure to carcinogens.

A DNA construct is introduced into the nuclei of embryonic stem cells. Cells containing the newly engineered genetic lesion are injected into a host mouse embryo, which is re-implanted into a recipient female. Some of these embryos develop into chimeric mice

that possess germ cells partially derived from the mutant cell line. Therefore, by breeding the chimeric mice it is possible to obtain a new line of mice containing the introduced genetic lesion (*see, e.g., Capecchi et al., Science* 244:1288 (1989)). Chimeric targeted mice can be derived according to Hogan *et al., Manipulating the Mouse Embryo: A Laboratory Manual*,
5 Cold Spring Harbor Laboratory (1988) and *Teratocarcinomas and Embryonic Stem Cells: A Practical Approach*, Robertson, ed., IRL Press, Washington, D.C., (1987).

Solid state and soluble high throughput assays

In one preferred embodiment, high throughput screening methods involve providing a combinatorial small organic molecule or peptide library containing a large
10 number of potential therapeutic compounds (potential modulator or ligand compounds). Such “combinatorial chemical libraries” or “ligand libraries” are then screened in one or more assays, as described herein, to identify those library members (particular chemical species or subclasses) that display a desired characteristic activity. The compounds thus identified can serve as conventional “lead compounds” or can themselves be used as potential
15 or actual therapeutics.

A combinatorial chemical library is a collection of diverse chemical compounds generated by either chemical synthesis or biological synthesis, by combining a number of chemical “building blocks” such as reagents. For example, a linear combinatorial chemical library such as a polypeptide library is formed by combining a set of chemical
20 building blocks (amino acids) in every possible way for a given compound length (*i.e.*, the number of amino acids in a polypeptide compound). Millions of chemical compounds can be synthesized through such combinatorial mixing of chemical building blocks.

Preparation and screening of combinatorial chemical libraries is well known to those of skill in the art. Such combinatorial chemical libraries include, but are not limited to,
25 peptide libraries (*see, e.g., U.S. Patent* 5,010,175, Furka, *Int. J. Pept. Prot. Res.* 37:487-493 (1991) and Houghton *et al., Nature* 354:84-88 (1991)). Other chemistries for generating chemical diversity libraries can also be used. Such chemistries include, but are not limited to: peptoids (*e.g., PCT Publication No.* WO 91/19735), encoded peptides (*e.g., PCT Publication No.* WO 93/20242), random bio-oligomers (*e.g., PCT Publication No.* WO 92/00091),
30 benzodiazepines (*e.g., U.S. Pat. No.* 5,288,514), diversomers such as hydantoins, benzodiazepines and dipeptides (Hobbs *et al., Proc. Nat. Acad. Sci. USA* 90:6909-6913 (1993)), vinylogous polypeptides (Hagihara *et al., J. Amer. Chem. Soc.* 114:6568 (1992)), nonpeptidal peptidomimetics with glucose scaffolding (Hirschmann *et al., J. Amer. Chem.*

Soc. 114:9217-9218 (1992)), analogous organic syntheses of small compound libraries (Chen *et al.*, *J. Amer. Chem. Soc.* 116:2661 (1994)), oligocarbamates (Cho *et al.*, *Science* 261:1303 (1993)), and/or peptidyl phosphonates (Campbell *et al.*, *J. Org. Chem.* 59:658 (1994)), nucleic acid libraries (*see* Ausubel, Berger and Sambrook, all *supra*), peptide nucleic acid
5 libraries (*see, e.g.*, U.S. Patent 5,539,083), antibody libraries (*see, e.g.*, Vaughn *et al.*, *Nature Biotechnology*, 14(3):309-314 (1996) and PCT/US96/10287), carbohydrate libraries (*see, e.g.*, Liang *et al.*, *Science*, 274:1520-1522 (1996) and U.S. Patent 5,593,853), small organic molecule libraries (*see, e.g.*, benzodiazepines, Baum C&EN, Jan 18, page 33 (1993); isoprenoids, U.S. Patent 5,569,588; thiazolidinones and metathiazanones, U.S. Patent
10 5,549,974; pyrrolidines, U.S. Patents 5,525,735 and 5,519,134; morpholino compounds, U.S. Patent 5,506,337; benzodiazepines, 5,288,514, and the like).

Devices for the preparation of combinatorial libraries are commercially available (*see, e.g.*, 357 MPS, 390 MPS, Advanced Chem Tech, Louisville KY, Symphony, Rainin, Woburn, MA, 433A Applied Biosystems, Foster City, CA, 9050 Plus, Millipore,
15 Bedford, MA). In addition, numerous combinatorial libraries are themselves commercially available (*see, e.g.*, ComGenex, Princeton, N.J., Asinex, Moscow, Ru, Tripos, Inc., St. Louis, MO, ChemStar, Ltd, Moscow, RU, 3D Pharmaceuticals, Exton, PA, Martek Biosciences, Columbia, MD, etc.).

In one embodiment the invention provides soluble assays using a reverse
20 transcriptase protein, or a cell or tissue expressing an reverse transcriptase, either naturally occurring or recombinant. In another embodiment, the invention provides solid phase based *in vitro* assays in a high throughput format, where the reverse transcriptase is attached to a solid phase. Any one of the assays described herein can be adapted for high throughput screening.

In the high throughput assays of the invention, either soluble or solid state, it is possible to screen up to several thousand different modulators or ligands in a single day. This methodology can be used for reverse transcriptase *in vitro*, or for cell-based or membrane-based assays comprising an reverse transcriptase protein. In particular, each well of a microtiter plate can be used to run a separate assay against a selected potential modulator, or,
25 if concentration or incubation time effects are to be observed, every 5-10 wells can test a single modulator. Thus, a single standard microtiter plate can assay about 100 (*e.g.*, 96) modulators. If 1536 well plates are used, then a single plate can easily assay from about 100-
30 about 1500 different compounds. It is possible to assay many plates per day; assay screens

for up to about 6,000, 20,000, 50,000, or more than 100,000 different compounds are possible using the integrated systems of the invention.

For a solid state reaction, the protein of interest or a fragment thereof, *e.g.*, an extracellular domain, or a cell or membrane comprising the protein of interest or a fragment thereof as part of a fusion protein can be bound to the solid state component, directly or indirectly, via covalent or non covalent linkage. A tag for covalent or non-covalent binding can be any of a variety of components. In general, a molecule which binds the tag (a tag binder) is fixed to a solid support, and the tagged molecule of interest is attached to the solid support by interaction of the tag and the tag binder.

A number of tags and tag binders can be used, based upon known molecular interactions well described in the literature. For example, where a tag has a natural binder, for example, biotin, protein A, or protein G, it can be used in conjunction with appropriate tag binders (avidin, streptavidin, neutravidin, the Fc region of an immunoglobulin, etc.) Antibodies to molecules with natural binders such as biotin are also widely available and appropriate tag binders; *see*, SIGMA Immunochemicals 1998 catalogue SIGMA, St. Louis MO).

Similarly, any haptenic or antigenic compound can be used in combination with an appropriate antibody to form a tag/tag binder pair. Thousands of specific antibodies are commercially available and many additional antibodies are described in the literature. For example, in one common configuration, the tag is a first antibody and the tag binder is a second antibody that recognizes the first antibody. In addition to antibody-antigen interactions, receptor-ligand interactions are also appropriate as tag and tag-binder pairs. For example, agonists and antagonists of cell membrane receptors (*e.g.*, cell receptor-ligand interactions such as transferrin, c-kit, viral receptor ligands, cytokine receptors, chemokine receptors, interleukin receptors, immunoglobulin receptors and antibodies, the cadherein family, the integrin family, the selectin family, and the like; *see, e.g.*, Pigott & Power, *The Adhesion Molecule Facts Book I* (1993). Similarly, toxins and venoms, viral epitopes, hormones (*e.g.*, opiates, steroids, etc.), intracellular receptors (*e.g.* which mediate the effects of various small ligands, including steroids, thyroid hormone, retinoids and vitamin D; peptides), drugs, lectins, sugars, nucleic acids (both linear and cyclic polymer configurations), oligosaccharides, proteins, phospholipids and antibodies can all interact with various cell receptors.

Synthetic polymers, such as polyurethanes, polyesters, polycarbonates, polyureas, polyamides, polyethyleneimines, polyarylene sulfides, polysiloxanes, polyimides,

and polyacetates can also form an appropriate tag or tag binder. Many other tag/tag binder pairs are also useful in assay systems described herein, as would be apparent to one of skill upon review of this disclosure.

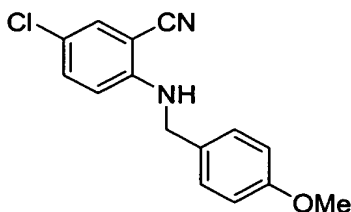
Common linkers such as peptides, polyethers, and the like can also serve as tags, and include polypeptide sequences, such as poly gly sequences of between about 5 and 200 amino acids. Such flexible linkers are known to persons of skill in the art. For example, poly(ethelyne glycol) linkers are available from Shearwater Polymers, Inc. Huntsville, Alabama. These linkers optionally have amide linkages, sulfhydryl linkages, or heterofunctional linkages.

Tag binders are fixed to solid substrates using any of a variety of methods currently available. Solid substrates are commonly derivatized or functionalized by exposing all or a portion of the substrate to a chemical reagent that fixes a chemical group to the surface, which is reactive with a portion of the tag binder. For example, groups that are suitable for attachment to a longer chain portion would include amines, hydroxyl, thiol, and carboxyl groups. Aminoalkylsilanes and hydroxyalkylsilanes can be used to functionalize a variety of surfaces, such as glass surfaces. The construction of such solid phase biopolymer arrays is well described in the literature. *See, e.g., Merrifield, J. Am. Chem. Soc.* 85:2149-2154 (1963) (describing solid phase synthesis of, *e.g.*, peptides); Geysen *et al., J. Immun. Meth.* 102:259-274 (1987) (describing synthesis of solid phase components on pins); Frank & Doring, *Tetrahedron* 44:60316040 (1988) (describing synthesis of various peptide sequences on cellulose disks); Fodor *et al., Science*, 251:767-777 (1991); Sheldon *et al., Clinical Chemistry* 39(4):718-719 (1993); and Kozal *et al., Nature Medicine* 2(7):753759 (1996) (all describing arrays of biopolymers fixed to solid substrates). Non-chemical approaches for fixing tag binders to substrates include other common methods, such as heat, cross-linking by UV radiation, and the like.

EXAMPLES

The following examples are offered to illustrate, but not to limit the claimed invention.

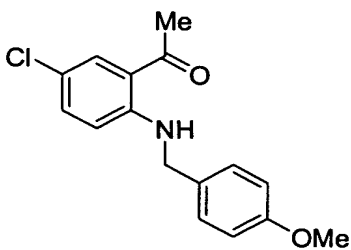
EXAMPLE 1



Preparation of 5-chloro-2-(4-methoxybenzylamino)benzonitrile (1)

5 A solution of 2-amino-5-chlorobenzonitrile (0.50 g, 3.28 mmol) in 2 mL of acetonitrile was treated with *p*-toluenesulfonic acid monohydrate. The reaction mixture was heated to 70 °C with stirring. 4-Methoxybenzyl alcohol (0.50, 3.61 mmol) in acetonitrile (1 mL) was added dropwise via syringe keeping the reaction temperature at 70 °C. The reaction was stirred at 70 °C for 12 h, allowed to cool to ambient temperature, and concentrated in
10 vacuo. Chromatography afforded **1** (0.884 g, 99%) as light yellow solid. ¹H NMR (CDCl₃, 400 MHz) δ 7.36 (d, *J* = 2.4 Hz, 1H), 7.27 (dd, *J* = 9 Hz, 1H), 7.24 (d, *J* = 8.6 Hz, 2H), 6.89 (d, *J* = 8.6 Hz, 2H), 6.58 (d, *J* = 9 Hz, 1H), 4.95 (br s, 1H), 4.33 (d, *J* = 5.4 Hz, 2H), 3.81 (s, 3H).

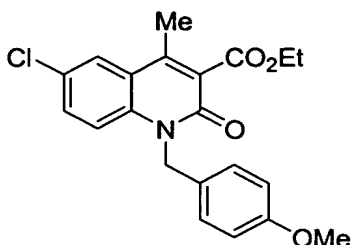
EXAMPLE 2



Preparation of 1-[5-chloro-2-(4-methoxybenzylamino)phenyl]ethanone (2)

15 A solution of **1** (0.40 g, 1.47 mmol) in 10 mL of anhydrous THF was cooled to 0 °C in an ice-water bath and treated with 1.2 mL of a 3.0 M solution of methylmagnesium chloride in THF. The reaction was allowed to warm to room temperature, stirred for 30 min, and acidified with aqueous 1N H₂SO₄. The reaction was extracted with EtOAc, dried
20 (Na₂SO₄), filtered, and concentrated in vacuo. Chromatography of the crude material gave **2** (0.35 g, 89%) as a crystalline yellow solid. ¹H NMR (CDCl₃, 400 MHz) δ 9.19 (br s, 1H), 7.69 (d, *J* = 1.3 Hz, 1H), 7.22 (m, 3H), 6.86 (d, *J* = 8.6 Hz, 2H), 6.61 (d, *J* = 9.1 Hz, 1H), 4.36
25 (d, *J* = 5.6 Hz, 2H), 3.81 (s, 3H), 2.57 (s, 3H).

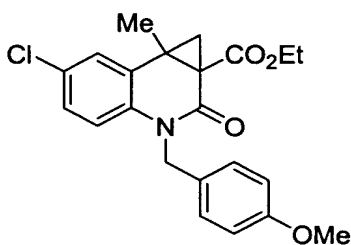
EXAMPLE 3



Preparation of 6-chloro-1-(4-methoxybenzyl)-4-methyl-2-oxo-1,2-dihydroquinoline-3-carboxylic acid ethyl ester (3)

A solution of **2** (0.20 g, 1.18 mmol) in 3 mL of anhydrous benzene was treated with ethyl-3-chloro-3-oxopropionate (165 μ L, 0.20 g, 1.30 mmol). The reaction mixture was heated to 80 $^{\circ}$ C for 1 h. The reaction was allowed to cool to room temperature and was concentrated in vacuo. The resulting oil was dissolved in 3 mL of ethanol, treated with NaOEt (40.0 mg, 0.59 mmol). The suspension was heated to 80 $^{\circ}$ C for 10 min, allowed to cool to room temperature, and diluted with H₂O. The resulting white precipitate was filtered to give **3** (0.38 g, 80%) as a white solid. ¹H NMR (CDCl₃, 400 MHz) δ 7.69 (d, J = 2.3 Hz, 1H), 7.39 (dd, J = 2.3, 9 Hz, 1H), 7.26 (d, J = 9 Hz, 1H), 7.15 (d, J = 8.6 Hz, 2H), 6.81 (d, J = 8.6 Hz, 2H), 5.47 (br s, 2H), 4.46 (q, J = 7.2 Hz, 2H), 3.75 (s, 3H), 2.46 (s, 3H), 1.43 (t, J = 7.2 Hz, 3H).

EXAMPLE 4

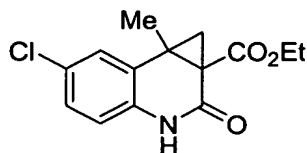


Preparation of 6-chloro-3-(4-methoxybenzyl)-7b-methyl-2-oxo-1,2,3,7b-tetrahydro-3-azacyclo-propa[a]naphthalene-1a-carboxylic acid ethyl ester (4)

A suspension of sodium hydride in anhydrous DMSO was treated in two portions with trimethylsulfoxonium iodide (0.33 g, 1.50 mmol). The reaction mixture was stirred for 30 min, treated with **3** (0.29 g, 0.752 mmol), and heated to 80 $^{\circ}$ C for 12 h. The resulting amber solution was allowed to cool to room temperature and poured over ice water. The milky suspension was extracted with CH₂Cl₂, dried (Na₂SO₄), filtered, and concentrated in vacuo. Chromatography provided **4** (0.22 g, 72%) as a white solid. ¹H NMR (CDCl₃, 400 MHz) δ 7.45 (d, J = 2.3 Hz, 1H), 7.13 (d, J = 8.6 Hz, 2H), 7.05 (dd, J = 2.2, 9 Hz, 1H), 6.84

(d, $J = 8.6$ Hz, 3H), 5.12 (m, 2H), 4.31 (m, 2H), 3.76 (s, 3H), 2.11 (d, $J = 6.6$ Hz, 1H), 1.58 (s, 3H), 1.31 (t, $J = 7.2$ Hz, 3H), 1.08 (d, $J = 6.6$ Hz, 1H).

EXAMPLE 5

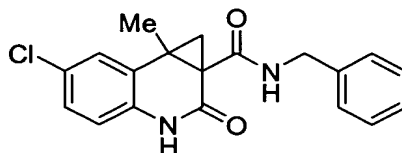


5

Preparation of 6-chloro-7b-methyl-2-oxo-1,2,3,7b-tetrahydro-3-aza-cyclopropa[a]naphthalene-1a-carboxylic acid ethyl ester (5)

A solution of **4** (20.0 mg, 0.050 mmol) in acetonitrile/H₂O (9:1, 0.300 mL) was cooled to 0 °C in an ice–water bath and treated with CAN (82.0 mg, 0.150 mmol). The reaction mixture was stirred at ambient temperature for 12 h and quenched with Na₂S₂O₅ to remove anisaldehyde. The resulting suspension was diluted with H₂O, extracted with CH₂Cl₂, dried (Na₂SO₄), filtered, and concentrated in vacuo. Chromatography afforded **5** (12.0 mg, 86%) as a white solid. ¹H NMR (CDCl₃, 400 MHz) δ 8.55 (br s, 1H), 7.43 (d, $J = 2.2$ Hz, 1H), 7.15 (dd, $J = 2.2, 9$ Hz, 1H), 6.72 (d, $J = 9$ Hz, 1H), 4.31 (m, 2H), 2.12 (d, $J = 6.6$ Hz, 1H), 1.55 (s, 3H), 1.29 (t, $J = 7.2$ Hz, 3H), 1.11 (d, $J = 6.6$ Hz, 1H).

EXAMPLE 6



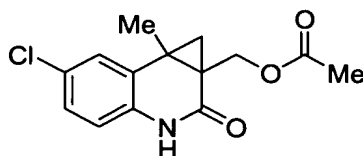
Preparation of 6-chloro-7b-methyl-2-oxo-1,2,3,7b-tetrahydro-3-aza-cyclopropa[a]naphthalene-1a-carboxylic acid benzyl amine (6)

A solution of **5** (14.0 mg, 0.050mmol) in THF/CH₃OH/H₂O (3:2:1, 800 μ L) was cooled to 0 °C in an ice–water bath and treated with 167 μ L of aq 3N LiOH (10 equiv). The cooling bath was removed, and the solution was stirred at 25 °C for 3 h. The solution was quenched with the addition of 1N KHSO₄, diluted with H₂O, and extracted with EtOAc. The combined organic phase was dried (Na₂SO₄), filtered, and concentrated in vacuo to give the desired carboxylic acid 9.4 mg, 75%) and a colorless oil. This material was dissolved in 800 μ L of CH₂Cl₂ and treated sequentially with benzyl amine (5.2 mg, 0.049 mmol), HOBT (5.1 mg, 0.037 mmol), EDCI (18.6 mg, 0.097 mmol). The reaction mixture was stirred for 12 h at 25 °C before the solvent was removed under a stream of N₂. Chromatography afforded **6**

(8.0 mg, 63%) as a white solid. ^1H NMR (CDCl_3 , 400 MHz) δ 9.22 (br s, 1H), 7.98 (br s, 1H), 7.48 (d, $J = 1.8$ Hz, 1H), 7.41 (d, $J = 7.3$ Hz, 2H), 7.31 (apparent t, $J = 7.1$ Hz, 2H), 7.20 (m, 2H), 6.95 (d, $J = 7.3$ Hz, 1H), 4.51 (m, 2H), 2.08 (d, $J = 6.6$ Hz, 1H), 1.52 (s, 3H), 1.02 (d, $J = 6.6$ Hz, 1H).

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EXAMPLE 7

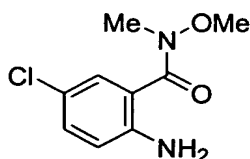


Preparation of acetic acid 6-chloro-7b-methyl-2-oxo-1,2,3,7b-tetrahydro-3-azacyclopropa[a]naphthalene-1a-ylmethyl ester (7)

10 A solution **5** (12.0 mg, 0.0429 mmol) in 500 μL anhydrous THF was treated with 47.2 mL of a 1.0 M solution of LiAlH_4 in diethyl ether. The reaction was stirred at 0°C for 1 h and carefully quenched with 100 μL of H_2O and 200 μL of saturated aq NH_4Cl . The reaction mixture was diluted with H_2O and extracted with EtOAc. The combined organic phase was dried (Na_2SO_4) filtered, and concentrated in vacuo to give the desired alcohol (4.7
15 mg, 46%) as a white solid. The alcohol was dissolved in CH_2Cl_2 , cooled to 0°C in an ice-water bath, and treated Et_3N (3.1 mg, 0.030 mmol), acetic anhydride (2.4 mg, 0.024 mmol), and catalytic DMAP. The reaction was allowed to warm to 25°C , diluted with H_2O , and extracted with EtOAc. The combined organics were dried (Na_2SO_4), filtered, and concentrated in vacuo. Chromatography afforded **7** (4.7 mg, 82%) as white solid. ^1H NMR
20 (CDCl_3 , 400 MHz) δ 9.29 (br s, 1H), 7.52 (d, $J = 1.8$ Hz, 1H), 7.19 (dd, $J = 2.2, 9$ Hz, 1H), 6.99 (d, $J = 9$ Hz, 1H), 5.15 (d, $J = 12$ Hz, 1H), 3.98 (d, $J = 712$ Hz, 1H), 1.95 (s, 3H), 1.77 (s, 3H), 1.75 (d, $J = 6.6$ Hz, 1H), 0.98 (d, $J = 6.6$ Hz, 1H).

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EXAMPLE 8

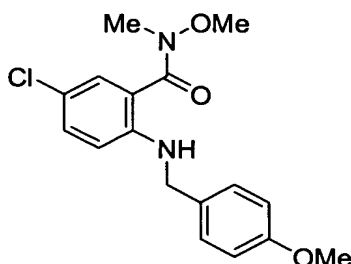


Preparation of 2-amino-5-chloro-N-methoxy-N-methylbenzamide (8)

A solution of 2-amino-5-chlorobenzoic acid (5.0 g, 29.1 mmol), *N,O*-dimethylhydroxylamine hydrochloride (3.1 g, 32.0 mmol), and HOBt (3.9 g, 29.1 mmol) in CH_2Cl_2 (100 mL) was cooled to 0°C and treated with EDCI (17.3 g, 87.3 mmol). The

reaction was stirred at 25 °C for 12 h, diluted with H₂O, and extracted with EtOAc. The organics were dried (Na₂SO₄), filtered, and concentrated. Chromatography (SiO₂, 1:1 Hex/EtOAc) gave **8** (3.7 g, 60%) as a white solid.

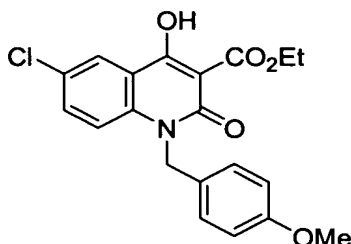
EXAMPLE 9



Preparation of 5-chloro-N-methoxy-2-(4-methoxy-benzylamino)-N-methyl-benzamide (9)

A solution of **8** (3.75 g, 17.3 mmol) in 50 mL of acetonitrile was treated with *p*-toluenesulfonic acid monohydrate. The reaction mixture was heated to 70 °C with stirring. 4-Methoxybenzyl alcohol (2.63 g, 19.0 mmol) in acetonitrile (3 mL) was added dropwise via syringe keeping the reaction temperature at 70 °C. The reaction was stirred at 70 °C for 12 h, allowed to cool to ambient temperature, and concentrated in vacuo. Chromatography afforded **9** (4.3 g, 75%) as light yellow solid.

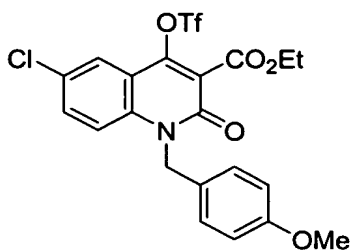
EXAMPLE 10



Preparation of 6-chloro-4-hydroxy-1-(4-methoxy-benzyl)-2-oxo-1,2-dihydro-quinoline-3-carboxylic acid ethyl ester (10)

A solution of **9** (0.13 g, 0.38 mmol) in 3 mL of anhydrous benzene was treated with ethyl-3-chloro-3-oxopropionate (54 µL, 63.0 mg, 0.421 mmol). The reaction mixture was heated to 80 °C for 1 h. The reaction was allowed to cool to room temperature and was concentrated in vacuo. The resulting oil was dissolved in 3 mL of ethanol, treated with NaOEt (40.0 mg, 0.59 mmol). The suspension was heated to 80 °C for 10 min, allowed to cool to room temperature, and diluted with H₂O. The resulting precipitate was filtered to give **10** (63.0 g, 43%) as a white solid.

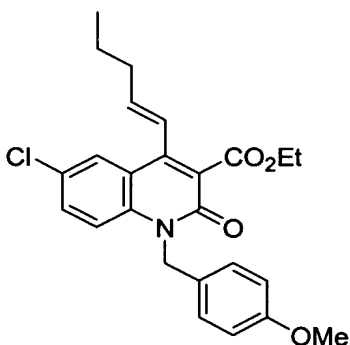
EXAMPLE 11



Preparation of 6-chloro-1-(4-methoxybenzyl)-2-oxo-4-(trifluoromethanesulfonyloxy)-1,2-dihydroquinoline-3-carboxylic acid ethyl ester (**11**)

A solution of **10** (63.0 mg, 0.16 mmol), DMAP (7.0 mg, 0.054 mmol), and diisopropylethylamine (19.0 mg, 0.15 mmol) in anhydrous CH₂Cl₂ was treated with trifluoromethylsulfonic acid (78.0 mg, 0.28 mmol). The reaction was stirred at room temperature for 30 min and concentrated. Chromatography (SiO₂, 3:1 Hex/EtOAc) gave **11** (75.0 mg, 88%) as a amorphous solid.

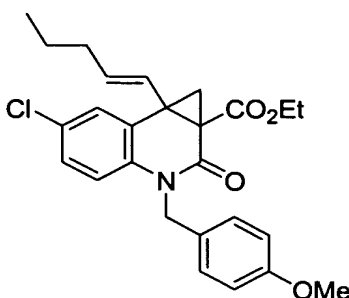
EXAMPLE 12



Preparation of 6-chloro-2-oxo-4-pent-1-enyl-1,2-dihydroquinoline-3-carboxylic acid ethyl ester (**12**)

A solution of **11** (0.10 g, 0.19 mmol), *E*-penten-1-ylboronic acid (44.0 mg, 0.38 mmol), tetrakis(triphenylphosphine)palladium (22.0 mg, 0.023 mmol), and NaHCO₃ (41.0 mg, 0.38 mmol) in 1:1 DME/H₂O (3 mL) was heated at 100 °C for 1 h. The reaction was allowed to cool to room temperature, diluted with H₂O, extracted with EtOAc, dried (Na₂SO₄), filtered, and concentrated. Chromatography (SiO₂, 3:1 Hex/EtOAc) gave **12** (43.0 mg, 51%) as a colorless film.

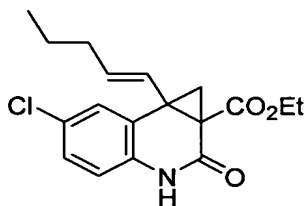
EXAMPLE 13



5 *Preparation of 6-Chloro-3-(4-methoxy-benzyl)-2-oxo-7b-pent-1-enyl-1,2,3,7b-tetrahydro-3-aza-cyclopropa[a]naphthalene-1a-carboxylic acid ethyl ester (13)*

A suspension of sodium hydride in anhydrous DMSO was treated in two portions with trimethylsulfoxonium iodide (65 mg, 0.29 mmol). The reaction mixture was stirred for 30 min, treated with **12** (43 mg, 0.098 mmol), and heated to 80 °C for 12 h. The resulting amber solution was allowed to cool to room temperature and poured over ice water.
10 The milky suspension was extracted with CH₂Cl₂, dried (Na₂SO₄), filtered, and concentrated in vacuo. Chromatography provided **13** (31 mg, 70%) as a colorless oil.

EXAMPLE 14



15 *Preparation of 6-chloro-2-oxo-7b-pent-1-enyl-1,2,3,7b-tetrahydro-3-aza-cyclopropa[a]naphthalene-1a-carboxylic acid ethyl ester (14)*

A solution of **13** (31 mg, 0.068 mmol) in acetonitrile/H₂O (9:1, 0.300 mL) was cooled to 0 °C in an ice–water bath and treated with CAN (112 mg, 0.204 mmol). The reaction mixture was stirred at ambient temperature for 12 h and quenched with Na₂S₂O₅ to
20 remove anisaldehyde. The resulting suspension was diluted with H₂O, extracted with CH₂Cl₂, dried (Na₂SO₄), filtered, and concentrated in vacuo. Chromatography afforded **14** (14 mg, 60%) as a white solid.

It is understood that the examples and embodiments described herein are for illustrative purposes only and that various modifications or changes in light thereof will be
25 suggested to persons skilled in the art and are to be included within the spirit and purview of this application and scope of the appended claims. All publications, patents, and patent

applications cited herein are hereby incorporated by reference in their entirety for all purposes.